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(54) Title: **SYNTHETIC METHODS FOR APLIDINE AND NEW ANTITUMORAL DERIVATIVES, METHODS OF MAKING AND USING THEM**

(57) Abstract: The invention provides aplidine derivatives and synthetic methods.

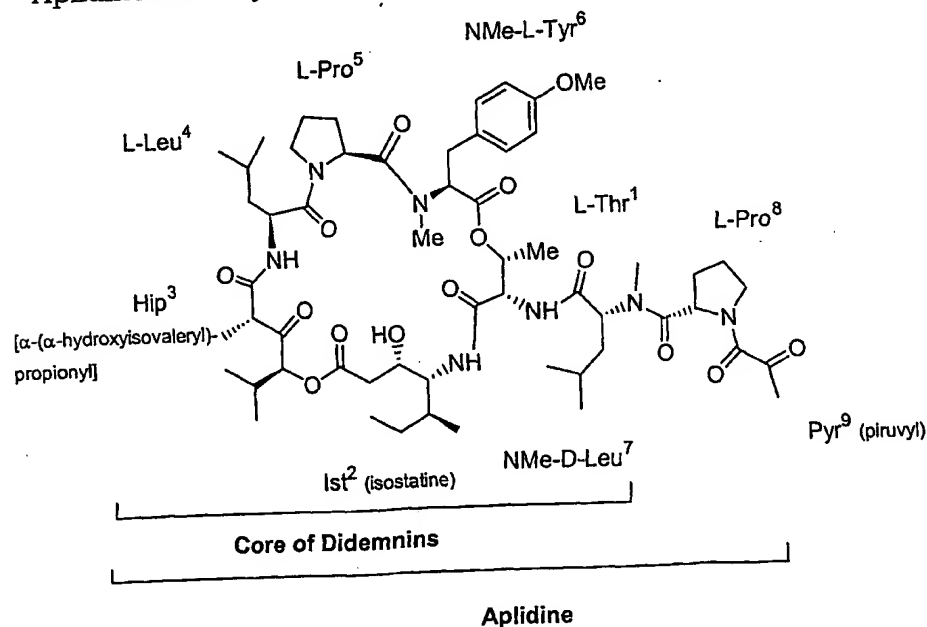
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## SYNTHETIC METHODS FOR APLIDINE AND NEW ANTITUMORAL DERIVATIVES, METHODS OF MAKING AND USING THEM.

The present invention relates to synthetic methods for aplidine and new antitumoral derivatives, methods of making and using them.

### BACKGROUND OF THE INVENTION

Aplidine has a cyclic structure with a sidechain, as follows:

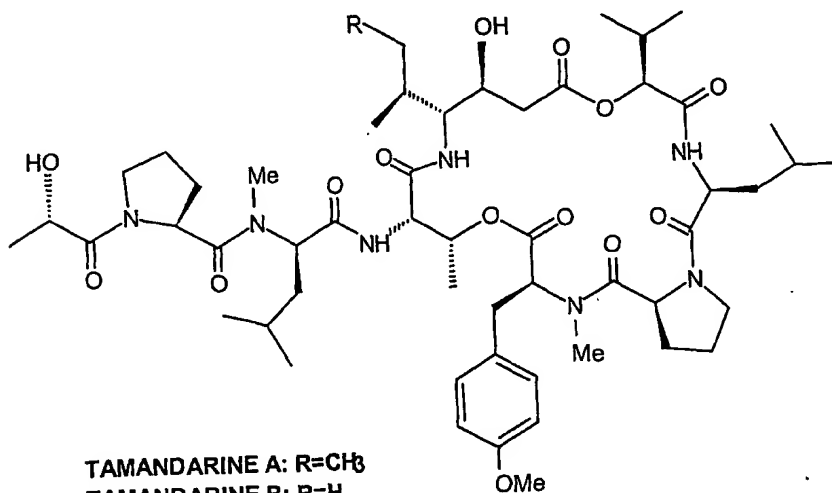


The didemnins form a class of cyclic depsipeptides which have been isolated from various species of the *Trididemnum* genus (Rinehart, Jr. et al. *J. Am. Chem. Soc.*, 103, 1857-59 (1981), Rinehart, Jr. et al. *Science*, 212, 933-935 (1981) with potent antitumoral and antiviral

activities. Among them, aplidine is one of the most antitumoral active natural didemnins. Description of the isolation and antitumoral activity of Aplidine is provided in US 5, 834, 586 patent.

A number of synthetic or natural analogs of Aplidine have been described (Rinehart, Jr. *et al. J. Med. Chem.*, **1996**, 39, 2819-2834) that include different modifications in the side chain, but preserving the same macrocyclic structure.

Recently have been described a related structure of didemnins called Tamandarins (Fenical, W. *et al., J. Org. Chem.*, **2000**, 65, 782-792) which were isolated from an unidentified ascidian of the family didemnidae. These molecules were found to differ only by the presence of hydroxyisovaleric acid (Hiv<sup>3</sup>), instead of the hydroxyisovalerylpropionic acid (Hip<sup>3</sup>). They have been described as highly active antiviral, antitumor and immunosuppressive peptides.



#### SUMMARY OF THE INVENTION

The present invention relates to the compounds described herein, termed aplidine derivatives for use in medicine, in particular in the treatment of tumours. The invention also relates to pharmaceutical preparations comprising them for treatment of tumours, for example, solid tumours, and use of the compound in the preparation of a medicament for the treatment of tumours. Treatment of solid tumours such as bladder, breast, colon, gastric, liver, nscl, ovary, pancreas, pharynx, prostate, renal, scl, retinoblastoma, melanoma, fibrosarcoma, chondrosarcoma, or osteosarcoma, or treatment of leukemia/lymphomas such as ALL (Promyelocytic leukemia), ALL (Acute lymphoblastic), CML (Chronic myelogenous), ALL (B-cell), leukemia (Hairy B-cell), leukemia (plasma cell), lymphoma (T cell), lymphoma (cutaneous T cell), lymphoma (undifferentiated), lymphoma (Burkitts B cell), lymphoma (histiocytic), lymphoma (B cell), lymphoma (Burkitts ascites) is particularly preferred.

Examples of pharmaceutical compositions of the invention include any solid (for example tablets, pills, capsules, granules) or liquid (solutions, suspensions or emulsions) with suitable composition or oral, topical or parenteral administration, and they may contain the pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

Suitably, the compound may be conjugated to a carrier protein or another suitable agent for delivery into the animal or human body. Conjugation may occur directly between a carrier and the compound, or indirectly via a suitable linker.

Administration of the compound or compositions of the present invention may be by any suitable method, such as intravenous infusion,



oral preparations, intraperitoneal and intravenous administration. We prefer that infusion times of up to 24 hours are used, more preferably 2-12 hours, with 2-6 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required. Infusion may be carried out at suitable intervals of say 2 to 4 weeks. Pharmaceutical compositions containing compounds of the invention may be delivered by liposome or nanosphere encapsulation, in sustained release formulations or by other standard delivery means.

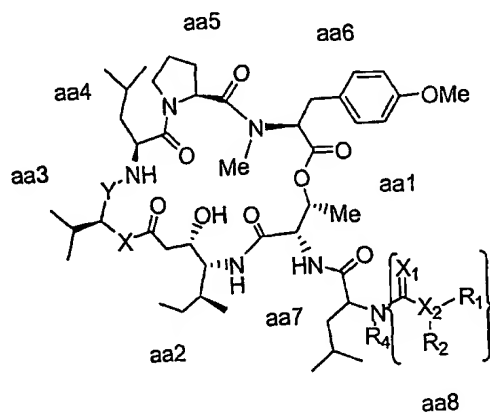
The correct dosage of the compound will vary according to the particular formulation, the mode of application, and the particular *situs*, host and cancer or tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The compounds of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

- a) drugs with antimetabolic effects, especially those which target cytoskeletal elements. including microtubule modulators such as taxane drugs (such as taxol, paclitaxel, taxotere, docetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);
- b) antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues such as pentostatin, methotrexate);

- c) alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosfamide);
- d) drugs which target DNA such as the anthracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;
- e) drugs which target such as etoposide; hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuporelin, goserelin, cyproterone or octreotide
- g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;
- h) alkylating drugs such as platinum drugs (cis-platin, carboplatin, oxaliplatin, paraptatin) or nitrosoureas;
- i) drugs potentially affecting metastasis of tumours such as matrix metalloproteinase inhibitors;
- j) gene therapy and antisense agents;
- k) antibody therapeutics; and
- l) other bioactive compounds of marine origin, notably the ecteinascidins such as ET-743.

In one aspect, the present invention relates to compounds of the formula:



wherein:

X is independently -CR<sub>2</sub>-, -O-, -S-, or -NR-, in which R is independently H or an organic group selected from an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and their substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group;

X<sub>2</sub> is independently CR, O (and R<sub>2</sub> is absent), S (and R<sub>2</sub> is absent), or N, in which R is independently H or an organic group selected from an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and their substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group;

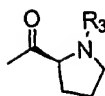
Y is -(COR')<sub>n</sub>CO-, where n is 0 or 1 and R' is an organic group selected from an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and their substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group;

X<sub>1</sub> is O or S;

R<sub>1</sub>, R<sub>2</sub> and R<sub>4</sub> are each independently H or an organic group selected from an amido group RCONH- or an acyl group RCO- where R is as defined, an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group, and R<sub>1</sub> or R<sub>2</sub> when X<sub>2</sub> is N, and R<sub>4</sub>, can further be -SO<sub>2</sub>R, where R is as defined;

or R<sub>1</sub> and R<sub>2</sub> with X<sub>2</sub> may form an optionally N-substituted proline, the N-substituted proline **aa8** being of formula

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where  $R_3$  is independently H or an organic group selected from a group  $RSO_2^-$  or an acyl group  $RCO^-$ , where R is as defined, or  $R_3$  is an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a carbonyl group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group;

or  $R_1$  and  $R_2$  with  $X_2$  may form a cycloalkyl, aryl or heterocyclic group, optionally substituted with one or more groups  $R_3$ ;

or  $R_1$ ,  $R_2$ ,  $X_2$ ,  $R_4$  and the nitrogen bearing  $R_4$  may form an oxadiazaspiroalkane N-substituted with  $R_5$ , where  $R_5$  is independently H or an organic group selected from a group  $RSO_2^-$  or an acyl group  $RCO^-$  where R is as defined, an alkyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a carbonyl group, an alkoxy group, an hydroxy group, a mercapto group, an amino group, a guanidino group, or a halogen group;

or **aa8** is replaced by an organic group selected from a group  $RSO_2^-$  or an acyl group  $RCO^-$  where R is as defined, an alkyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a carbonyl group, an alkoxy group, an hydroxy group, a mercapto group, an amino group, a guanidino group, or a halogen group; and pharmaceutically acceptable salts thereof.

Preferred compounds include those wherein X is  $-NR-$ , in which R is as defined. More preferably, X is  $-NH-$  or  $-NMe-$ , and most preferably X is  $-NH-$ .

Further preferred compounds include those wherein X is  $-O-$ .

The group Y is preferably -COR'CO-, where R' is an alkyl group, especially where R' is -CHCH<sub>3</sub>-.

Further preferred compounds include those wherein Y is -CO-.

In view of these preferences, a preferred class of compounds is that wherein X is --NH- or -O- and Y is -COCHCH<sub>3</sub>CO- or -CO-.

Preferably R<sub>4</sub> is methyl.

Preferably X<sub>1</sub> is =O.

Preferably X<sub>2</sub>R<sub>1</sub> is an optionally substituted aralkyloxy group, such as a benzyloxy group.

Other preferred compounds include those wherein X<sub>2</sub>R<sub>1</sub> is an optionally substituted amino group, more preferably those wherein X<sub>2</sub>R<sub>1</sub> is a group -NHR<sub>1</sub>, where R<sub>1</sub> is an optionally substituted alkyl group, alkenyl group, aryl group, or aralkyl group, especially an alkyl group or an aryl group, such as a phenyl group or a butyl group.

Further preferred compounds comprise those wherein X<sub>2</sub>R<sub>1</sub> is an optionally substituted alkyl group, especially where X<sub>2</sub>R<sub>1</sub> is a propyl group, isopropyl group, pentyl group or biotin group.

A group of preferred compounds is those wherein -C(=X<sub>2</sub>)R<sub>1</sub>R<sub>2</sub> form an optionally substituted amino acid acyl group. Suitably the optionally substituted amino acid acyl group is optionally substituted proline or optionally substituted glycine or optionally substituted valine, and more especially the optionally substituted proline is optionally substituted norvaline-proline, optionally substituted alanine-proline, Boc-proline,

optionally substituted alkyl-proline, or the optionally substituted glycine is heterocyclic-substituted glycine, or the optionally substituted valine is valine, Boc-valine, or alkyl-valine. Preferably the optionally substituted proline is norvaline-proline, Z-norvaline-proline, alanine-proline, Z-alanine-proline, Boc-alanine-proline, isobutyrylproline or optionally protected D-lactylproline, or the heterocyclic-substituted glycine is coumarinyl-glycine, or the optionally substituted valine is valine, Boc-valine, or isobutyrylvaline.

A further group of preferred compounds includes those wherein  $X_1$  is S and  $X_2R_1$  is a group  $-NHR_1$ , where  $R_1$  is an optionally substituted alkyl group, alkenyl group, aryl group, or aralkyl group.  $R_1$  is preferably an alkyl group or an aryl group, more preferably a phenyl group or a butyl group.

$R_1$  and  $R_2$  with  $X_2$  can form a heterocyclic group, optionally substituted with one or more groups  $R_3$ . For example, the heterocyclic group can be coumarin.

Preferred compounds include those wherein **aa8** is replaced by an organic group  $RSO_2^-$ , where R is as defined, such as methyl.

$R_1$ ,  $R_2$ ,  $X_2$ ,  $R_4$  and the nitrogen bearing  $R_4$  can form an oxadiazaspiroalkane N-substituted with  $R_5$ , where  $R_5$  is H. The N-substituted oxadiazaspiroalkane is preferably 6-oxa-1,7-diazaspiro[4,4]nonane.

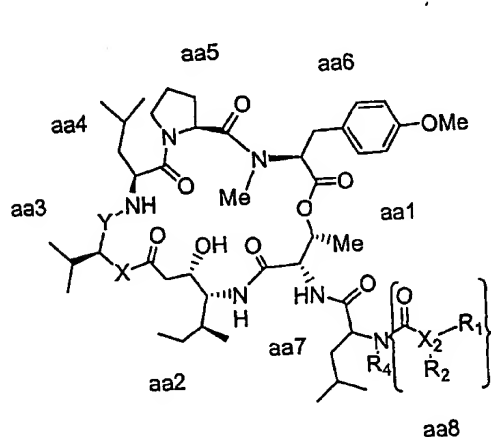
Examples of compounds according to this invention include:

- 3-[Aip]-Z-didemnin A,
- 8-[Phenylurea]-didemnin A,
- 8-[Butylurea]-didemnin A,

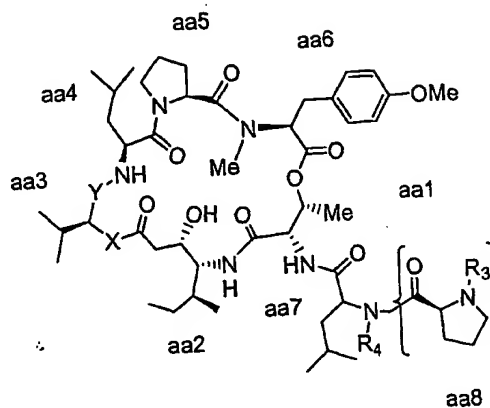
3-[val]-8-[isobutyryl]-aplidine,  
9-[norvaline]-aplidine,  
3-[Hiv]-9-[Isobutyryl]-aplidine,  
3-[Val]-9-[Isobutyryl]-aplidine,  
3-[hiv]-8-[isobutyryl]-didemnin A,  
3-[Hiv]-9-[Ala]-aplidine,  
3-[Hiv]-9-[Nva]-aplidine,  
8-[Phenylthiourea]-didemnin A,  
8-[Coumarin]-didemnin A,  
8-[Butylthiourea]-didemnin A,  
3-[Hiv]-9-[D-Lac]-aplidine ,  
8-[Methylsulphonyl]-didemnin A,  
3-[val]-Z-didemnin A,  
3-[Hiv]-8-[Val]-didemnin A,  
3-[Hiv]-8-[butyryl]-aplidine,  
3-[val]-didemnin A,  
3-[Hiv]-didemnin A,  
Z-Didemnin A,  
9-[Z-Nva]-aplidine,  
3-[Hiv]-9-[Z-ala]-aplidine,  
8-[Gly]-9-[Coumarin]-didemnin A,  
8-[Biotin]-didemnin A,  
3-[Hiv]-7,8-[Spiro]-9-[Boc]-aplidine,  
3-[Hiv]-Z-didemnin A,  
3-[Hiv]-9-[Z-Nva]-aplidine,  
7,8-[Spiro]-9-[pyr]-aplidine,  
3-[Hiv]-9-[lac(OTBDMS)]-aplidine,  
3-[Hiv]-9-[Boc-Ala]-aplidine,  
7,8-[Spiro]-9-[Boc]-aplidine,  
3-[Hiv]-8-[Boc-Val]-aplidine,  
8-[Val]-9-[Isobutyryl]-didemnin A,

3-[Hiv]-8-[hexanoyl]-didemnin A,  
 3-[Val]-9-[Lac(OTBDMS)]-aplidine,  
 3-[Aip]-didemnin A,  
 3-[Hiv]-9-[D-Lac(OTBDMS)]-aplidine,  
 7,8-[Spiro]-9-[Isobutyryl]-aplidine,  
 3-[Hiv]-7,8-[Spiro]-9-[Pyr]-aplidine,  
 3-[Hiv]-7,8-[Spiro]-9-[Isobutyryl]-aplidine,  
 3-[Hiv]-7,8-[Spiro]-9-[Acryloyl]-aplidine, or  
 [Aip]<sup>3</sup>-aplidine.

In a related aspect, the present invention is directed to compounds having the following formulae:



**Formula I**



**Formula II**

and related structures.

In one particularly preferred embodiment, the present invention provides a synthetic route to the formation of aa3 = [Hiv]<sup>3</sup> or [Val]<sup>3</sup> or [Aip]<sup>3</sup>, as a part of a series of exceedingly potent and rare antitumor agents which scheduled slated for clinical trials when adequate



quantities become available, and its simplest isomers, where amino acid residues are permuted. This process is enantio- and stereocontrolled and fast, taking advantages of the standard methods of solution-phase synthetic methodology

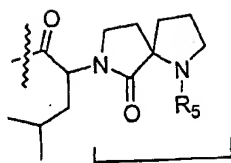
The preferred embodiment of the present invention is represented in formula I, wherein **aa3** are independently  $\alpha$ -amino acids of L or D configuration. If applies **X** is independently C, O, S, or NR; where R is independently H or an organic group selected from the group consisting of an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto group, an amino group, a guanidino group, a halogeno group. Where R most preferably have from 1 to about 12 carbon atoms, more preferably 1 to about 8 carbon atoms, still more preferably 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups in the compounds of the present invention. Where **aa3** most preferably is  $\alpha$ -( $\alpha'$ -hydroxyisovaleryl)propionyl (Hip), (X= O, Y= -COCHCH<sub>3</sub>CO-) -serie A, or  $\alpha$ -( $\alpha'$ -aminoisovaleryl)propionyl (Aip) (X= NH, Y= -COCHCH<sub>3</sub>CO-) -serie N, or valine (X = NH, Y= -CO-) -serie V, or  $\alpha$ -hydroxyisovaleryl (X = -O-, Y = -CO-) -serie H, or N-methylvaline (X= NMe, Y = -CO-) -serie M. Wherein **aa8** are independently  $\alpha$ -amino acids of L or D configuration, if applies; wherein **X<sub>2</sub>** is independently C, O, S, or N an organic group selected from the group consisting of an alkenyl, an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto independently H or an organic group selected from the group consisting of an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto group, an amino group, a guanidino group, a halogeno group, wherein R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub> and R<sub>4</sub> are each independently H or an organic group selected from the

group consisting of an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto group, an amino group, a guanidino group, a halogen group. **Aa8** also can be a proline residue as in formula II. Where  $R_3$  is independently H or an organic group selected from the group consisting of an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto group, an amino group, a guanidino group, a halogen group.

Where  $R_3$  most preferably can be pyruvic acid, aralkyloxycarbonyl group or aminoacid or peptides. Alkyl groups preferably have from 1 to about 12 carbon atoms, more preferably 1 to about 8 carbon atoms, still more preferably 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups in the compounds of the present invention. As used herein, the term alkyl, unless otherwise modified, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members. Preferred aminoacids are protected or non protected D or L glycine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, methionine, cysteine, aspartate, asparagine, glutamic acid, glutamine, lysine, arginine, proline, serine, threonine, histidine and hydroxyproline. Preferred peptides can be formed with the above mentioned aminoacids.

Besides, **aa8** and  $R_4$  can be linked through derivatives of a 6-oxo-1,7-diazaspiro[4,4]-nonane structure:

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6-oxa-1,7-diazaspiro  
[4,4]nonane derivatives

where  $R_5$  most preferably can be pyruvic acid, aralkyloxycarbonyl group or amino acid or peptides. Alkyl groups preferably have from 1 to about 12 carbon atoms, more preferably 1 to about 8 carbon atoms, still more preferably 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms. Methyl, ethyl and propyl including isopropyl are particularly preferred alkyl groups in the compounds of the present invention. As used herein, the term alkyl, unless otherwise modified, refers to both cyclic and noncyclic groups, although cyclic groups will comprise at least three carbon ring members. Preferred amino acids are protected or non protected D or L glycine, valine, leucine, isoleucine, phenylalanine, tyrosine, tryptophan, methionine, cysteine, aspartate, asparagine, glutamic acid, glutamine, lysine, arginine, proline, serine, threonine, histidine and hydroxyproline. Preferred peptides can be formed with the above mentioned amino acids.

As used herein, the term "organic group" means a hydrocarbon group that is classified as an aliphatic group, cyclic group, or combination of aliphatic and cyclic groups (*e.g.*, aralkyl groups). In the context of the present invention, the term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example. The term "alkyl group" means a saturated linear or branched hydrocarbon group including, for example, methyl, ethyl, isopropyl, isobutyl, *t*-butyl, heptyl, docetyl, octadecyl, amyl, 2-ethylhexyl, 2-methylbutyl, 5-

methylnonyl, and the like. The term "alkenyl group" means an unsaturated, linear or branched hydrocarbon group with one or more carbon-carbon double bonds, such as a vinyl group. The term "alkynyl group" means an unsaturated, linear or branched hydrocarbon group with one or more carbon-carbon triple bonds. The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group. The term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups. The term "aromatic group" or "aryl group" means a mono- or polycyclic aromatic hydrocarbon group. The term "heterocyclic group" means a closed ring hydrocarbon in which one or more of the atoms in the ring is an element other than carbon (e.g., nitrogen, oxygen, sulfur, etc.).

Preferred alkoxy groups in the compounds of the present invention include groups having one or more oxygen linkages and from 1 to about 12 carbon atoms, more preferably from 1 to about 8 carbon atoms, and still more preferably 1 to about 6 carbon atoms, and most preferably 1, 2, 3 or 4 carbon atoms.

Suitable heteroaromatic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., coumarinyl including 8-coumarinyl, quinolinyl including 8-quinolinyl, pyridyl, pyrazinyl, pyrimidyl, furyl, pyrrolyl, thienyl, thiazolyl, oxazolyl, imidazolyl, indolyl, benzofuranyl and benzothiazol. Suitable heteroalicyclic groups in the compounds of the present invention contain one, two or three heteroatoms selected from N, O or S atoms and include, e.g., tetrahydrofuranyl, tetrahydropyranyl, piperidinyl, morpholino and pyrrolidinyl groups.

Suitable carbocyclic aryl groups in the compounds of the present invention include single and multiple ring compounds, including multiple ring compounds that contain separate and/or fused aryl groups. Typical carbocyclic aryl groups contain 1 to 3 separate or fused rings and from 6 to about 18 carbon ring atoms. Specifically preferred carbocyclic aryl groups include phenyl including substituted phenyl, such as 2-substituted phenyl, 3-substituted phenyl, 2,3-substituted phenyl, 2,5-substituted phenyl, 2,3,5-substituted and 2,4,5-substituted phenyl, including where one or more of the phenyl substituents is an electron-withdrawing group such as halogen, cyano, nitro, alkanoyl, sulfinyl, sulfonyl and the like; naphthyl including 1-naphthyl and 2-naphthyl; biphenyl; phenanthryl; and anthracyl.

Optionally protected amino groups can be protected using groups known for this purpose. Suitable protecting groups for amines include carbamates, amides, and other protecting groups, such as alkyl, arylalkyl, sulfo- or halo- arylalkyl, haloalkyl, alkylsilylalkyl, arylalkyl, cycloalkylalkyl, alkylarylalkyl, heterocyclalkyl, nitroarylalkyl, acylaminoalkyl, nitroaryldithioarylalkyl, dicycloalkylcarboxamidoalkyl, cycloalkyl, alkenyl, arylalkenyl, nitroarylalkenyl, heterocyclalkenyl, heterocycl, hydroxyheterocycl, alkylidithio, alkoxy- or halo- or alkylsulphinyl arylalkyl, heterocyclacyl, and other carbamates, and alkanoyl, haloalkanoyl, arylalkanoyl, alkenoyl, heterocyclacyl, aroyl, arylaroyl, haloaroyl, nitroaroyl, and other amides, as well as alkyl, alkenyl, alkylsilylalkoxyalkyl, alkoxyalkyl, cyanoalkyl, heterocycl, alkoxyarylalkyl, cycloalkyl, nitroaryl, arylalkyl, alkoxy- or hydroxyarylalkyl, and many other groups. Such groups may optionally be substituted with the previously mentioned substituent groups.

References herein to substituted groups in the compounds of the present invention refer to the specified moiety that may be substituted at

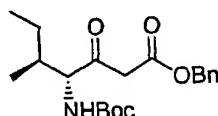
one or more available positions by one or more suitable groups, e.g., halogen such as fluoro, chloro, bromo and iodide; cyano; hydroxyl; nitro; azido; alkanoyl such as a C1-6 alkanoyl group such as acyl and the like; carboxamido; alkyl groups including those groups having 1 to about  $\infty$  2 carbon atoms or from 1 to about 6 carbon atoms and more preferably 1-3 carbon atoms; alkenyl and alkynyl groups including groups having one or more unsaturated linkages and from 2 to about 12 carbon or from 2 to about 6 carbon atoms; alkoxy groups having those having one or more oxygen linkages and from 1 to about 12 carbon atoms or 1 to about 6 carbon atoms; aryloxy such as phenoxy; alkylthio groups including those moieties having one or more thioether linkages and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; alkylsulfinyl groups including those moieties having one or more sulfinyl linkages and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; alkylsulfinyl groups including those moieties having one or more sulfonyl linkages and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; aminoalkyl groups such as groups having one or more N atoms and from 1 to about 12 carbon atoms or from 1 to about 6 carbon atoms; carbocyclic aryl having 6 or more carbons, particularly phenyl (e.g., R being a substituted or unsubstituted biphenyl moiety); and aralkyl such as benzyl.

As is well understood in this technical area, a large degree of substitution is not only tolerated, but is often advisable. Substitution is anticipated on the compounds of the present invention. As a means of simplifying the discussion and recitation of certain terminology used throughout this application, the terms "group" and "moiety" are used to differentiate between chemical species that allow for substitution or that may be substituted and those that do not allow or may not be so substituted. Thus, when the term "group" is used to describe a chemical substituent, the described chemical material includes the

unsubstituted group and that group with O, N, or S atoms, for example, in the chain as well as carbonyl group or other conventional substitution. Where the term "moiety" is used to describe a chemical compound or substituent, only a unsubstituted chemical material is intended to be included. For example, the phrase "alkyl group" is intended to include not only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, isobutyl, and the like, but also alkyl substituents bearing further substituents known in the art, such as hydroxy, alkoxy, amino, carboxyl, carboxamido, halogen atoms, cyano, nitro, alkylsulfonyl, etc. Thus, "alkyl group" includes ether groups, haloalkyls, alcohols, thiols, carboxyl, amines, hydroxyalkyls, sulfoalkyls, etc. On the other hand, the phrase "alkyl moiety" is limited to the inclusion of only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, isobutyl, and the like.

In a further aspects of this invention, there are provided synthetic methods.

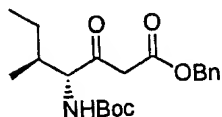
A method is provided of making a didemninn fragment having the structure



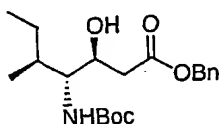
the method comprising coupling Boc-D-allo-Ileu-OH with the lithium enolate of benzyl acetate.

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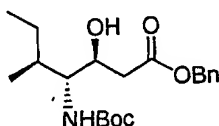
The carbonyl group of a didemninn fragment of formula:



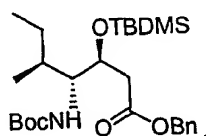
can be reduced to yield a didemninn fragment having the structure



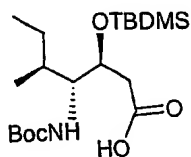
The hydroxy group of a compound of formula:



can be protected to yield a didemninn fragment having the structure

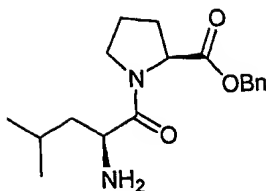


Further deprotection of the benzyl ester group yields a didemninn fragment having the structure

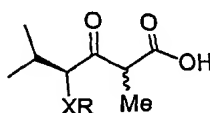


A further method of this invention for making a didemninn fragment comprises coupling a first reactant having the structure

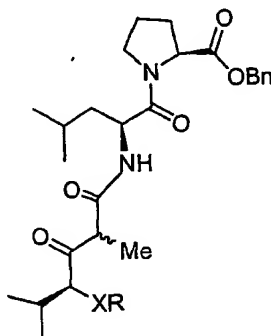




and a second reactant having the structure

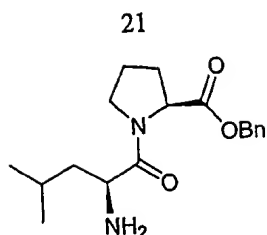


to yield a didemninn fragment having the structure

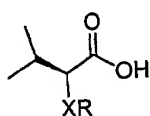


wherein X is selected from the group consisting of -O- and -NH-; where R is an amine protecting group; and where R is a hydroxy protecting group. Suitably X is -O- and R is tert-butyldimethylsilyl; or X is -NH- and R is Boc.

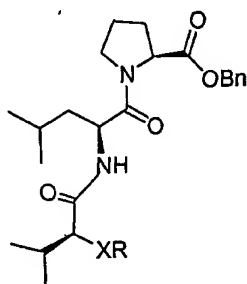
Another method of this invention for making a didemninn fragment comprises coupling a first reactant having the structure



and a second reactant having the structure

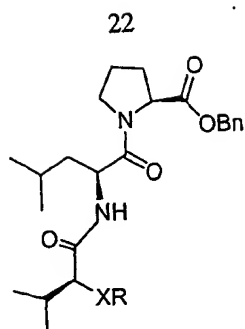


to yield a didemninn fragment having the structure

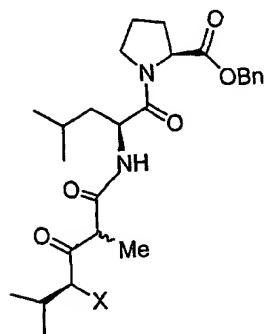


wherein X is selected from the group consisting of -O-, -NMe, and -NH-; where R is an amine protecting group; and where R is H. Suitably X is -O- and R is H.; or X is -NH- and R is Boc; or X is -NMe- and R is Boc.

A method of this invention comprises hydrolyzing the didemninn fragment of formula:

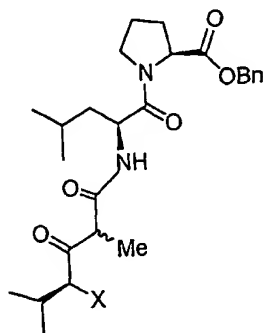


to yield a didemninn fragment having the structure

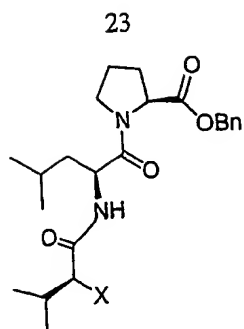


wherein X is selected from the group consisting of  $-OH$ , and  $-NH_2$

Another method involves hydrolyzing the didemninn fragment

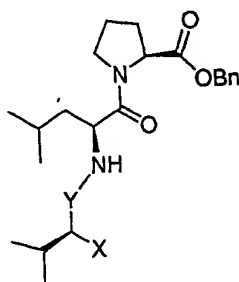


to yield a didemninn fragment having the structure

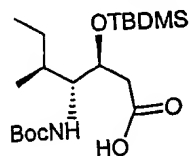


wherein X is selected from the group consisting of  $-NH_2$  and  $-NHMe$ .

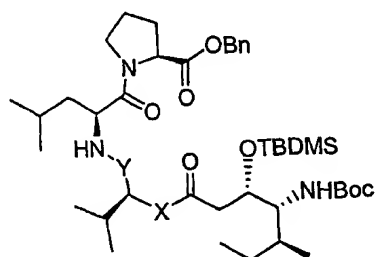
A further method is provided of making a didemninn fragment, the method comprising coupling a first reactant having the structure



and a second reactant having the structure



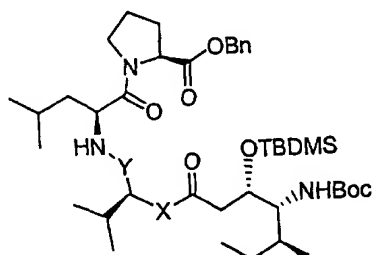
to yield a didemninn fragment having the structure



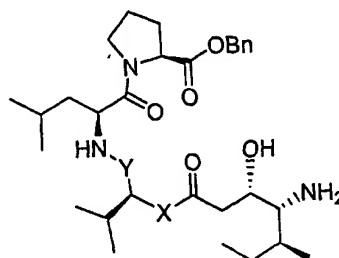
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wherein X is selected from the group consisting of -O-, -NMe, and -NH-;  
 where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

A method comprises comprising hydrolyzing the didemninn fragment

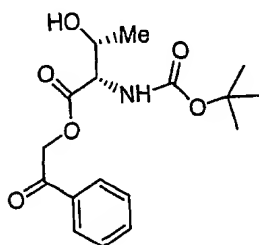


to yield a didemninn fragment having the structure

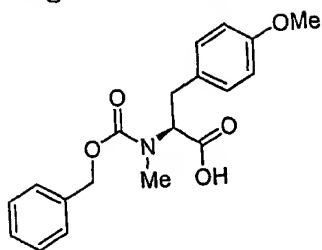


wherein X is selected from the group consisting of -O-, and -NH-; where  
 Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

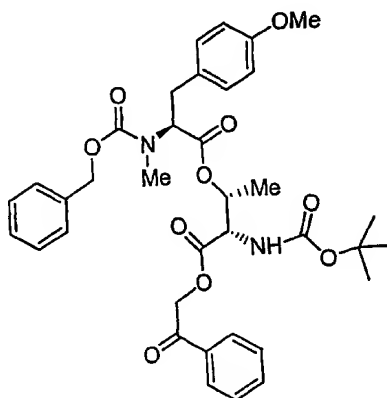
Another method of making a didemninn fragment is provided by this  
 invention, the method comprising coupling a first reactant having the  
 structure



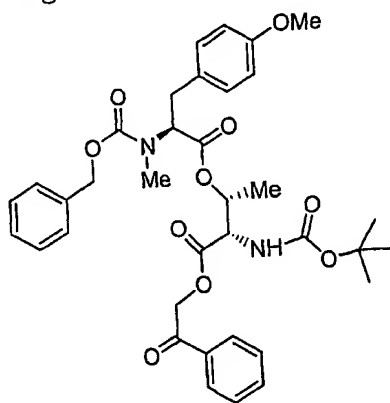
and a second reactant having the structure



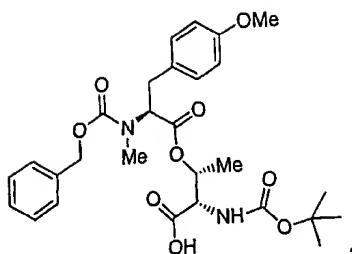
to yield a didemninn fragment having the structure



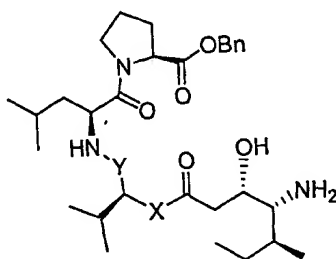
A method of this invention involves deprotection of the benzyl ester group of the didemninn fragment of formula



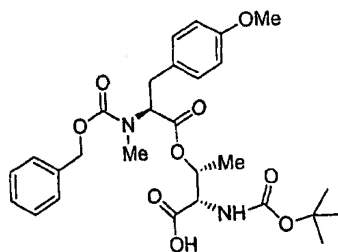
to yield a didemninn fragment having the structure



According to this invention, a method of making a didemninn fragment comprises coupling a first reactant having the structure

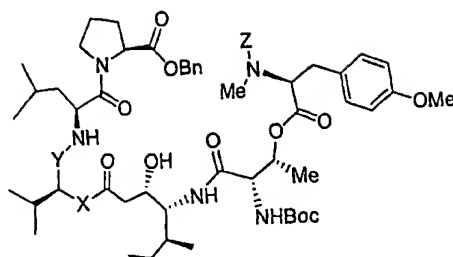


and a second reactant having the structure



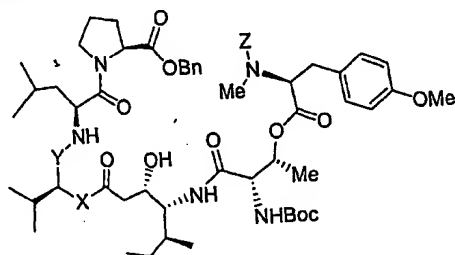
to yield a didemninn fragment having the structure

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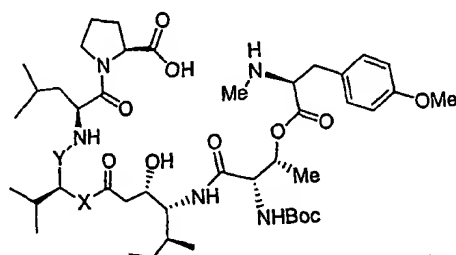


wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

A method comprises deprotection of the didemninn fragment



to yield a didemninn fragment having the structure

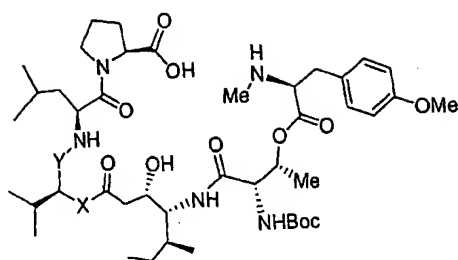


wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

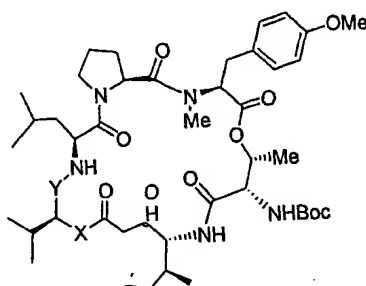
A further method of this invention for making a didemninn fragment comprises the cyclizing the fragment of formula:



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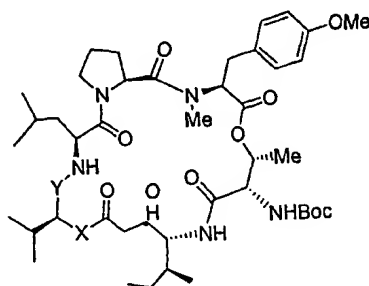


to yield a didemnins analog having the structure



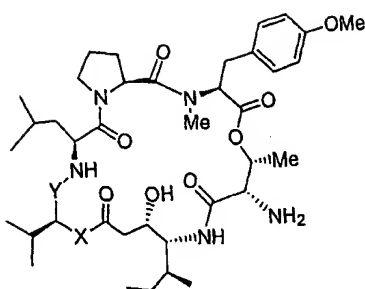
wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

A method involves hydrolyzing the didemnins analog



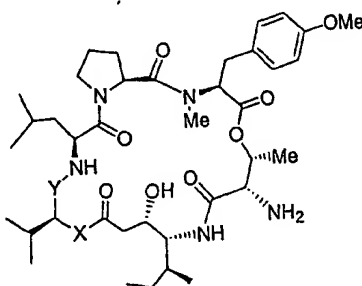
to yield a didemnins analog having the structure

29

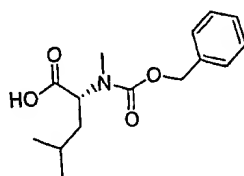


wherein X is selected from the group consisting of  $-O-$ , and  $-NH-$ ; where Y is  $-(COCHCH_3)_nCO-$ ; where n is 0 or 1.

A method is further provided of making a didemnins analog, the method comprising coupling a first reactant having the structure

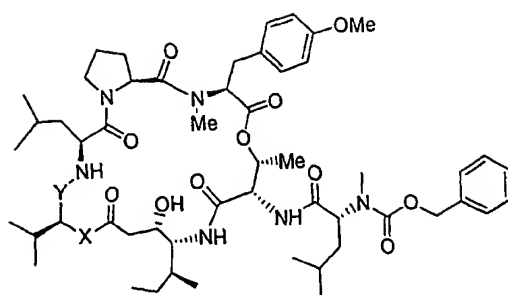


and a second reactant having the structure



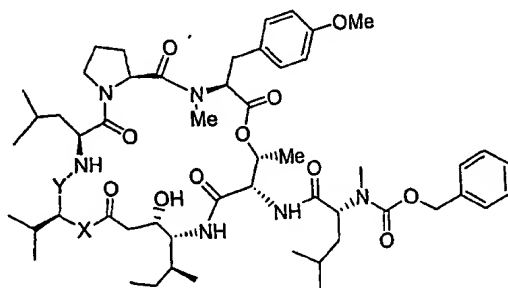
to yield a didemnins analog having the structure

30

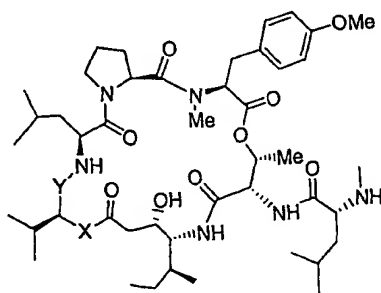


wherein X is selected from the group consisting of  $-O-$ , and  $-NH-$ ; where Y is  $-(COCHCH_3)_nCO-$ ; where n is 0 or 1.

Another method comprises deprotection the didemninn fragment of formula:



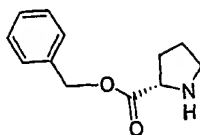
to yield a didemninn fragment having the structure



wherein X is selected from the group consisting of  $-O-$ , and  $-NH-$ ; where Y is  $-(COCHCH_3)_nCO-$ ; where n is 0 or 1.

31

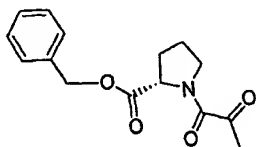
A method of making a didemninn fragment comprises the coupling of the fragment having the structure



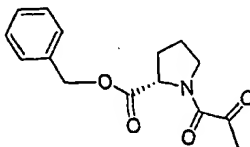
and a second reactant having the structure



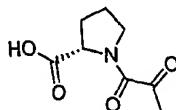
to yield a didemninn fragment having the structure



A further method comprises deprotection the didemninn fragment of formula:

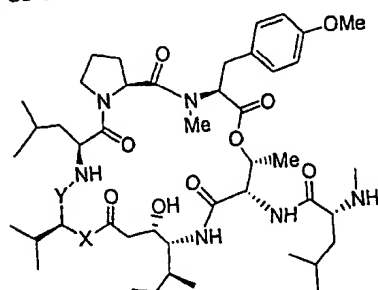


to yield a didemninn fragment having the structure

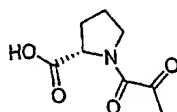


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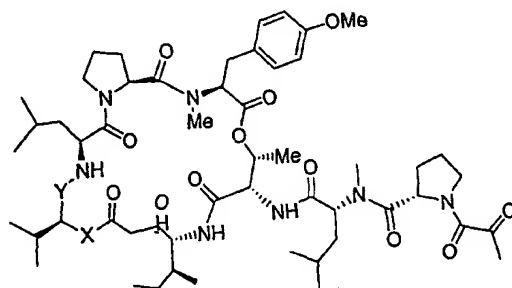
A method of this invention for making a didemninn analog comprises the coupling of the didemninn analog of formula:



with the fragment



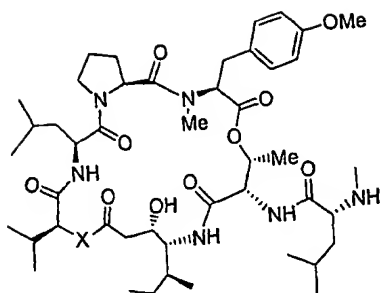
to yield the didemninn analog having the structure



wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

A method of making a didemninn analog comprises the coupling of the didemninn analog having the structure

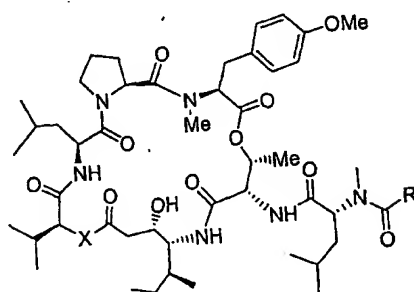
33



and the fragment having the structure



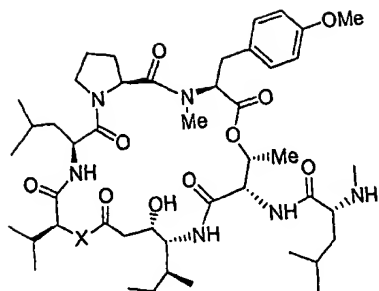
to yield the didemninn analog having the structure



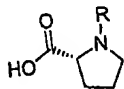
wherein X is selected from the group consisting of -O-, and -NH-, and R is i-Propyl; wherein X is -O- and R is n-Propyl, and R is n-Pentyl

A method of this invention for making a didemninn analog comprises the coupling of the didemninn analog having the structure

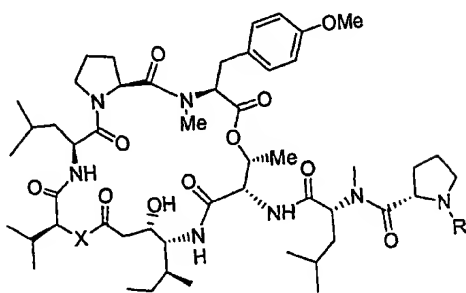
34



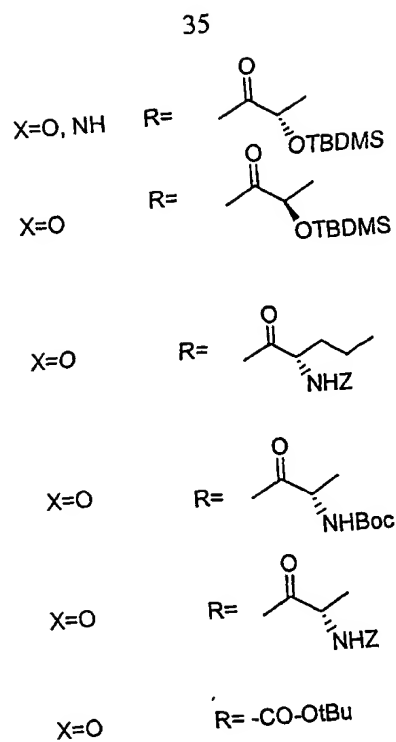
and the fragment having the structure



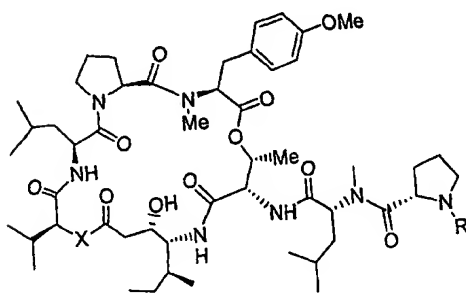
to yield the didemnin analog having the structure



wherein :



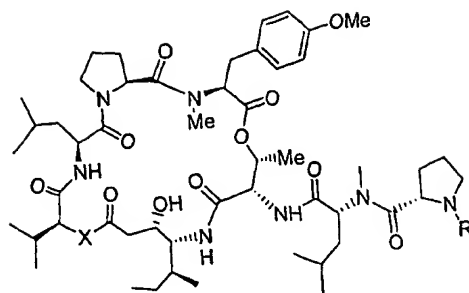
A method of this invention involves deprotection the didemninn analog



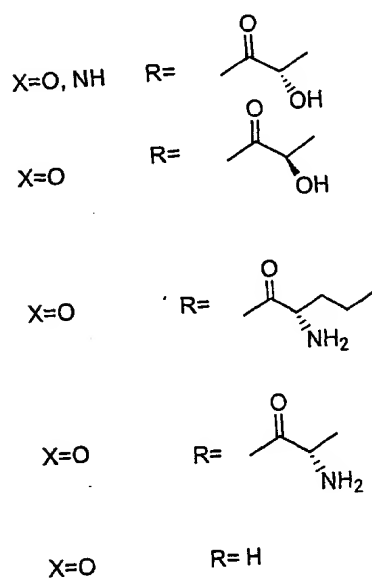
to yield a didemninn fragment having the structure



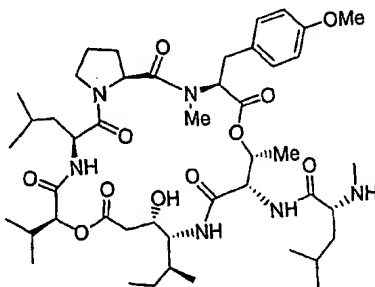
36



wherein

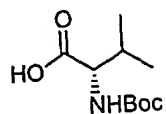


A method of making a didemnin analog is provided comprising the coupling of the didemnin analog having the structure

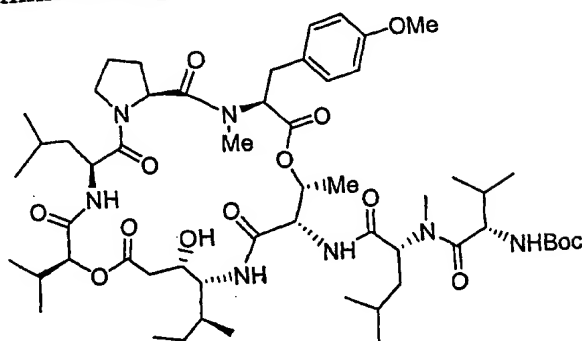


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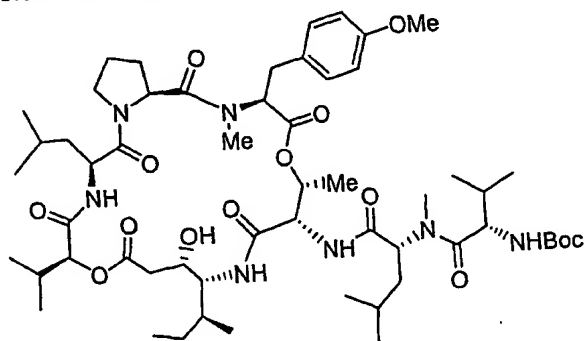
and the fragment having the structure



to yield the didemninn analog having the structure

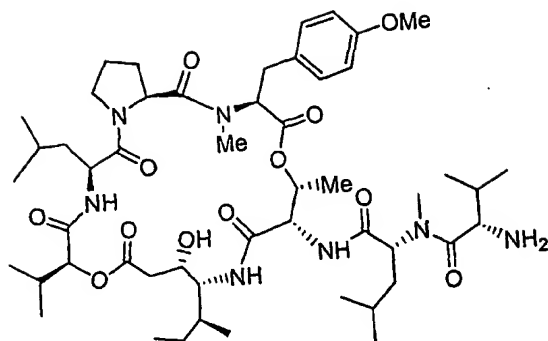


A method further comprises deprotection the didemninn analog

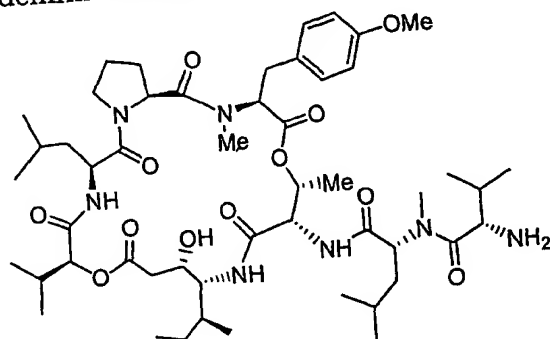


to yield a didemninn analog having the structure

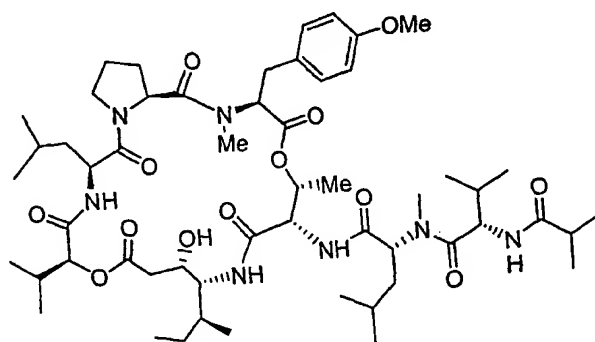
38



A method is provided of making a didemninn analog comprising the coupling of the didemninn analog

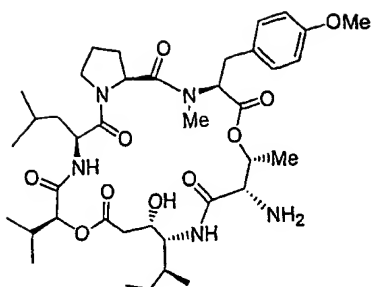


and isobutyryl chloride to yield the didemninn analog having the structure

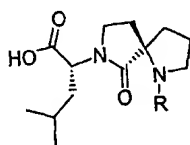


A method of making a didemninn analog is provided comprising the coupling of the didemninn analog having the structure

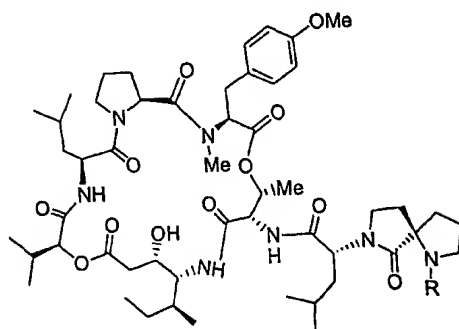
39



the fragment having the structure



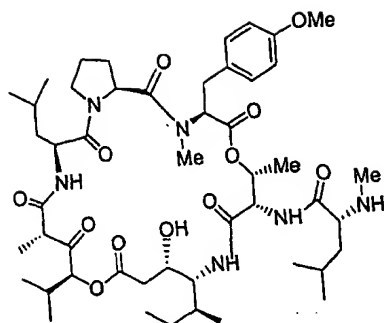
to yield the didemnins analog having the structure



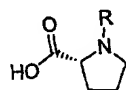
wherein R is Boc, isobutyryl, pyruvyl, or acryloyl.

A method of making a didemnins analog comprises the coupling of the didemnins analog having the structure

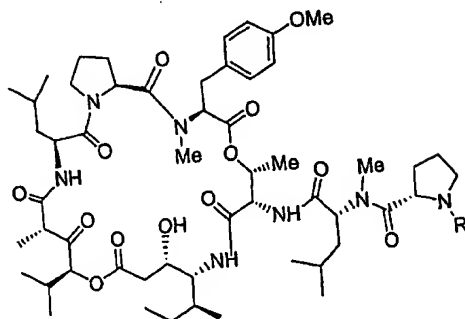
40



and the fragment having the structure

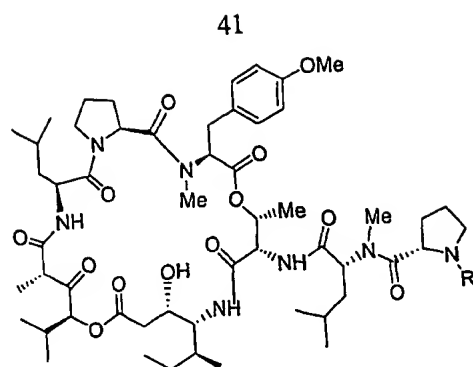


to yield the didemnins analog having the structure

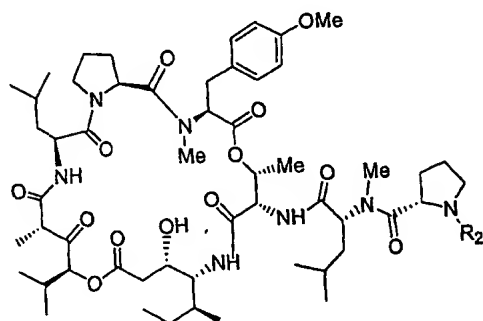


wherein R is SO<sub>2</sub>Me, or Z-Nva.

A method is provided by this invention comprising deprotection the didemnins analog

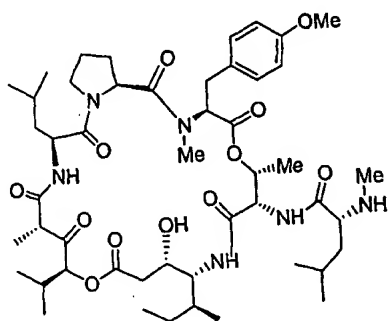


to yield a didemninn analog having the structure



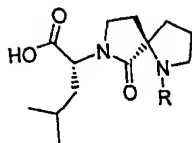
wherein R<sub>2</sub> is Nva.

A method of making a didemninn analog is part of this invention, comprising the coupling of the didemninn analog having the structure

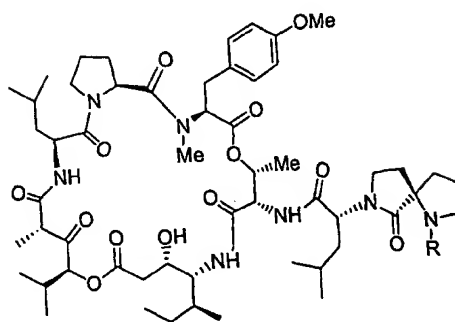


and the fragment having the structure

42

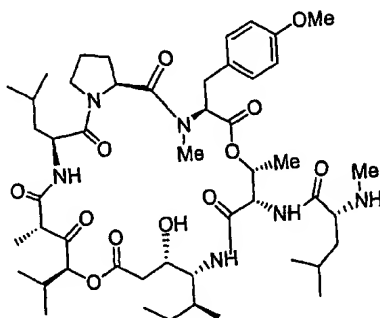


to yield the didemninn analog having the structure

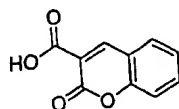


wherein R is Boc, isobutyryl, or pyruvyl.

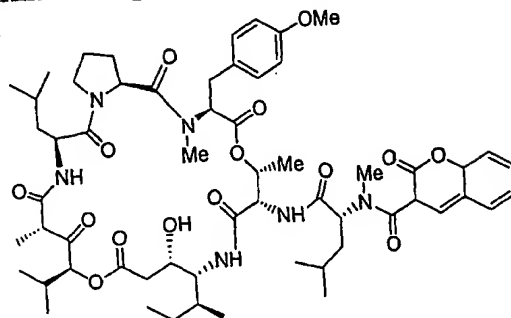
A method of making a didemninn analog comprises the coupling of the didemninn analog having the structure



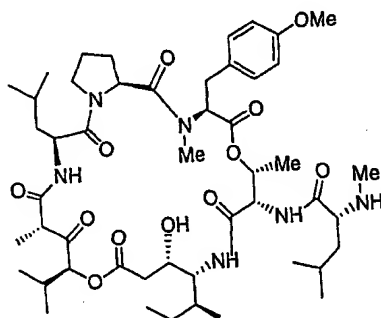
and the fragment having the structure



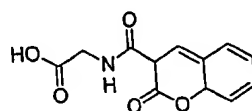
to yield the didemninn analog having the structure



A method of making a didemninn analog is provided comprising the coupling of the didemninn analog having the structure



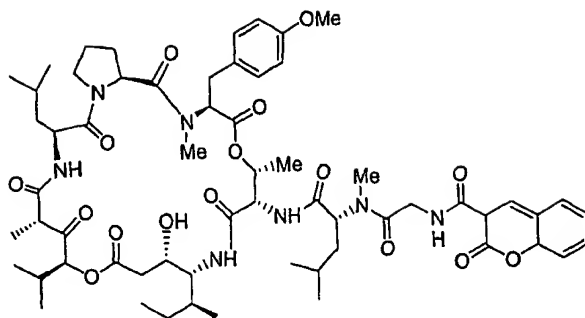
and the fragment having the structure



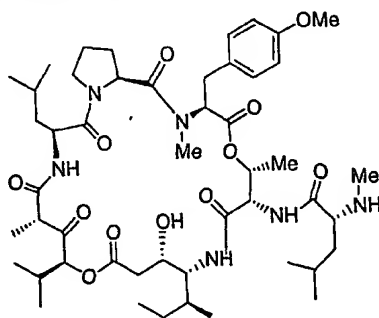
to yield the didemninn analog having the structure



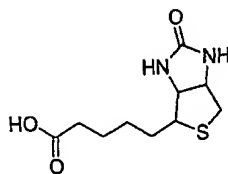
44



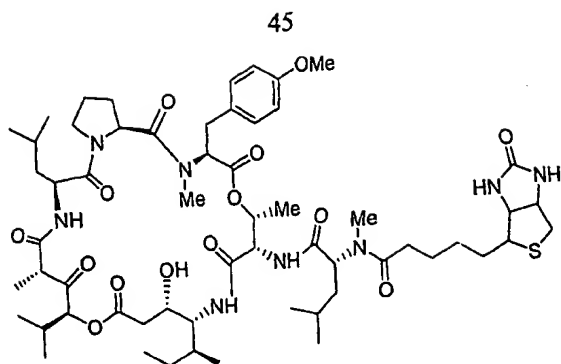
A method of making a didemnyn analog comprising the coupling of the didemnyn analog having the structure



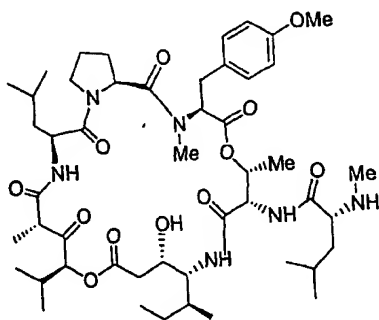
and the fragment having the structure



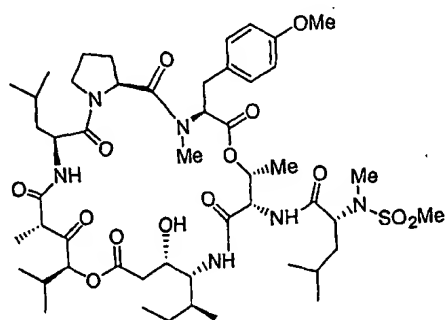
to yield the didemnins analog having the structure



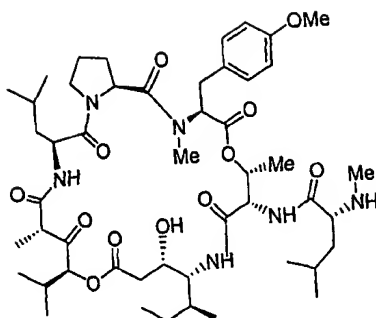
A method of making a didemninn analog is provided comprising the coupling of the didemninn analog having the structure



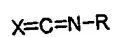
and methylsulphonyl chloride, to yield the didemninn analog having the structure



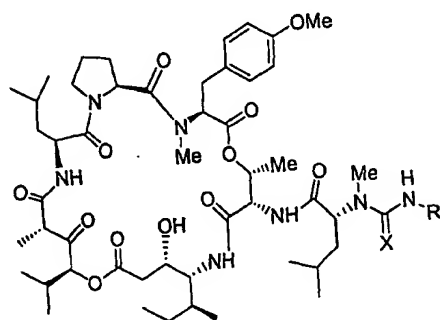
A method of making a didemninn analog comprises the coupling of the didemninn analog having the structure



and the fragment having the structure



to yield the didemnin analog having the structure



wherein X is O, and S; wherein R is butyl, and phenyl.

It will be appreciated that these methods are all illustrative of the present invention and can be modified as desired. In particular, different protecting groups can be adopted for protection of amino groups or hydroxy groups. Different reagents can be employed to introduce intended groups. The substituents may be varied as desired, with particular regard to the general formula for the compounds of this

invention, and the examples of preferred meanings. The modified methods are part of this invention.

To the extent that it may be necessary to ensure that this description includes all of the disclosure in our priority applications, and to ensure entitlement to the full extent to the priority dates, we hereby incorporate by reference the content of our GB 0016148.9 and GB 0103750.6.

#### DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The compounds of the present invention can be prepared synthetically. The methods described here for the synthesis of aplidine and derivatives can also be used for the synthesis of a broad range of didemnins.

The structures of some of the compounds are shown in Figure 1:

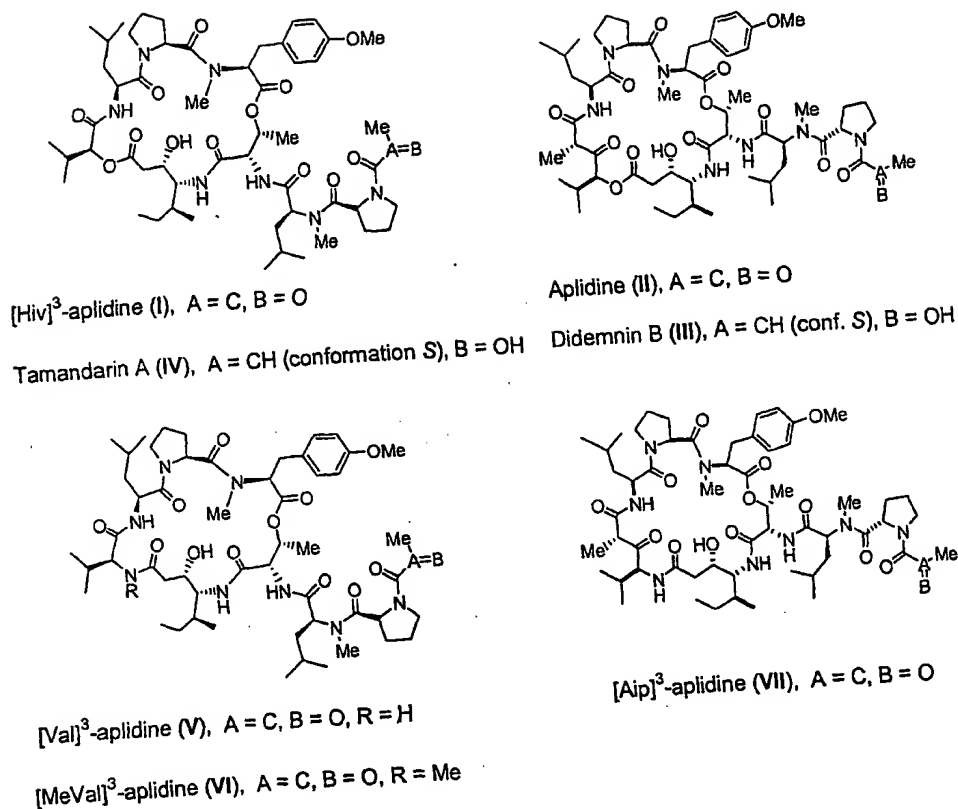


Figure 1

Aa3-aplidine derivatives are synthetic cyclic depsipeptides similar in structure to aplidine (compound II) (aa3 = Hip, also known as dehydrodidemnin B) which is a natural didemnin isolated from the ascidian *Aplidium albicans* (Figure 1). The molecules prepared differs by the presence of hydroxyisovaleric acid (Hiv) (compound I), or valine (compound V) or methyl valine (compound VI) or  $\alpha$ -( $\alpha$ -aminoisovaleryl)propionyl (Aip) (compound VII) instead of the hydroxyisovalerylpropionic (Hip) (II) unit which is present in all other naturally occurring didemnin congeners. The similarity between these two structures has also been found recently between didemnin B (compound III), the most well-known member of this class of depsipeptides, and a new isolated cyclic depsipeptide from an unidentified Brazilian ascidian

of the family Didemnidae: Tamandarine A (compound **IV**). Compounds **I**, **V**, **VI** and **VII** are non natural didemnin derivatives.

The structural homology between **III** and **IV** is also reflected in their respective biological activity. Comparing both compounds, **IV** retains similar levels of in vitro antitumor activity in clonogenic assays as well as protein biosynthesis inhibition properties, and it has been shown to be somewhat more active in vitro than **III** against pancreatic carcinoma. However, compound **IV** does not show any tumor type specificity whatsoever in NCI 60 cell panel. Didemnin B proved to be toxic at doses near those required for therapeutic applications and it is likely that **IV** is a broad spectrum toxin rather than a selective agent.

[Hiv]<sup>3</sup>-aplidine (**I**) otherwise exhibits the same benefits found in apolidine (**II**) with respect to didemnin B (**III**), in that is more specific against solid tumors like colon, chondrosarcoma and osteosarcoma. in the MTS assay. [Val]<sup>3</sup>-aplidine (**V**) and [MeVal]<sup>3</sup>-aplidine (**VI**) are otherwise new compounds which exhibit a high level of in vitro antitumor activity. Finally, compounds **V**, **VI** and **VII** are likely to result, with respect to the parent apolidine, in an increase in hydrogen bonding at the active site, and thus, provide more active compounds. In addition the presence of the amide bond replacing the ester bond may improve the stability of the cyclodepsipeptide core.

We report here the first total synthesis of the different series of apolidine derivatives. By way of example the retrosynthetic analysis is shown in Figure 2.

The key steps include an efficient macrocyclization of linear precursors **6**, and a practical stereoselective synthesis of Ist-aa3-Leu-Pro unit (**A1**), and the right fragment **D1**. Final coupling of the macrocycle **4**

with different side chains affords aa3-aa8-aplidine and derivatives. The robustness of this synthetic methodology has been proved successfully in developing a practical synthesis of aplidine **II** (aa3= Hip).

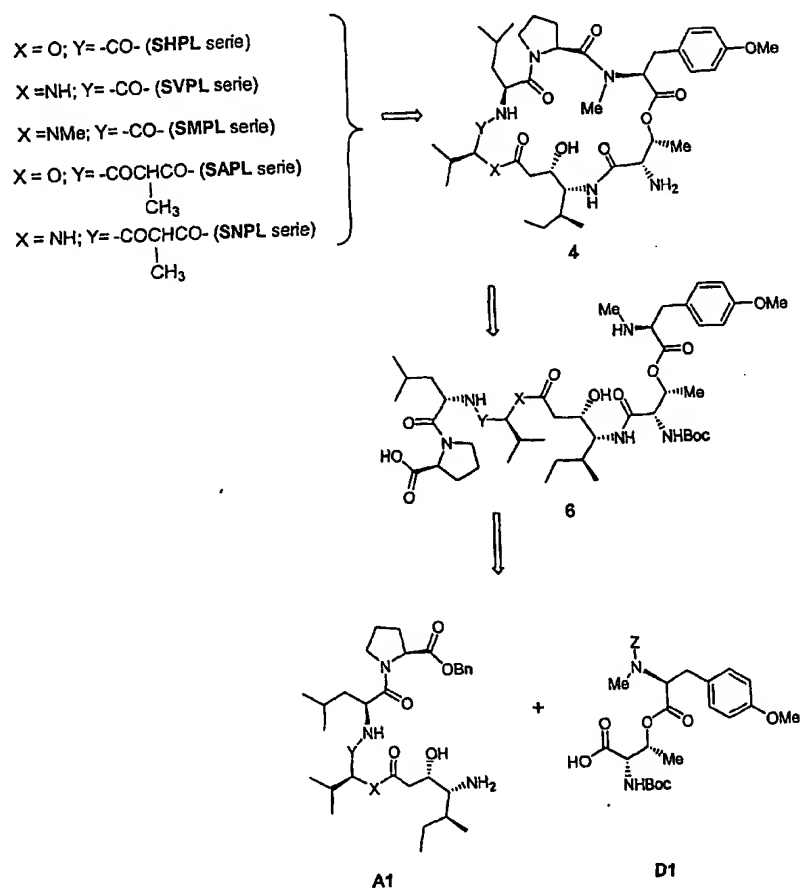
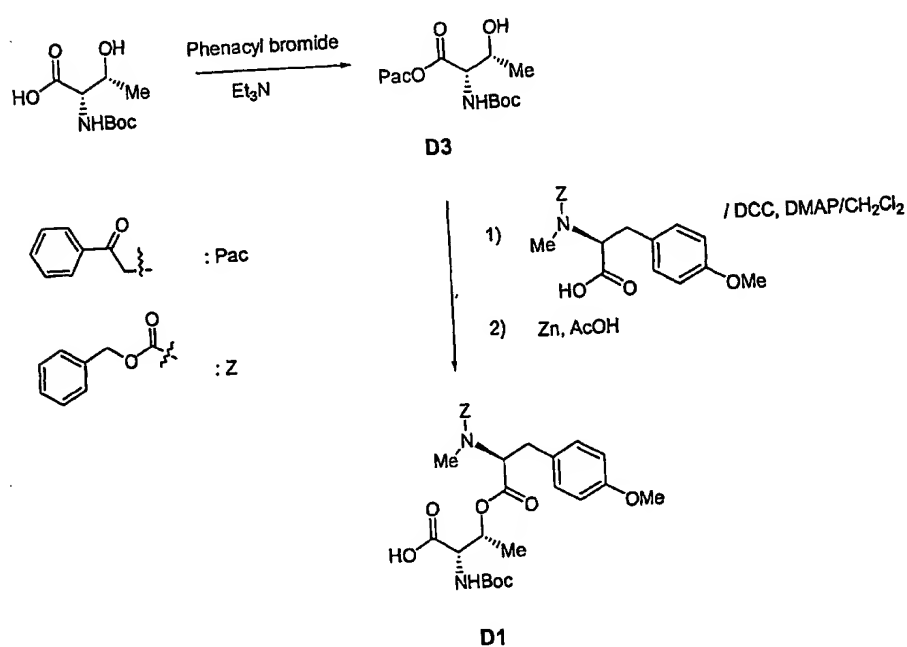


Figure 2

The formation of the macrocyclic core is the essential key step in all series. Successful cyclization at all of the four possible amide bonds has been achieved in previous syntheses of the didemnins. However, in the present work, the bond linking N(Me)-O(Me)-Tyr and Pro was selected

as the point for macrocyclization based on previous work developed during the total synthesis of aplidine **II** (G. Jou, I. Gonzalez, F. Albericio, P. Lloyd-Williams, E. Giralt., *J. Org. Chem.* **1997**, 62, 354-366 and patent ES-2102322).



Scheme 1

The macrocyclic core of the target molecule is disconnected into Ist-aa3-Leu-Pro tetrapeptide left unit **A1** and the dipeptide Boc-Thr-(Z-NMe-OMe-Tyr)-OH **D1**.

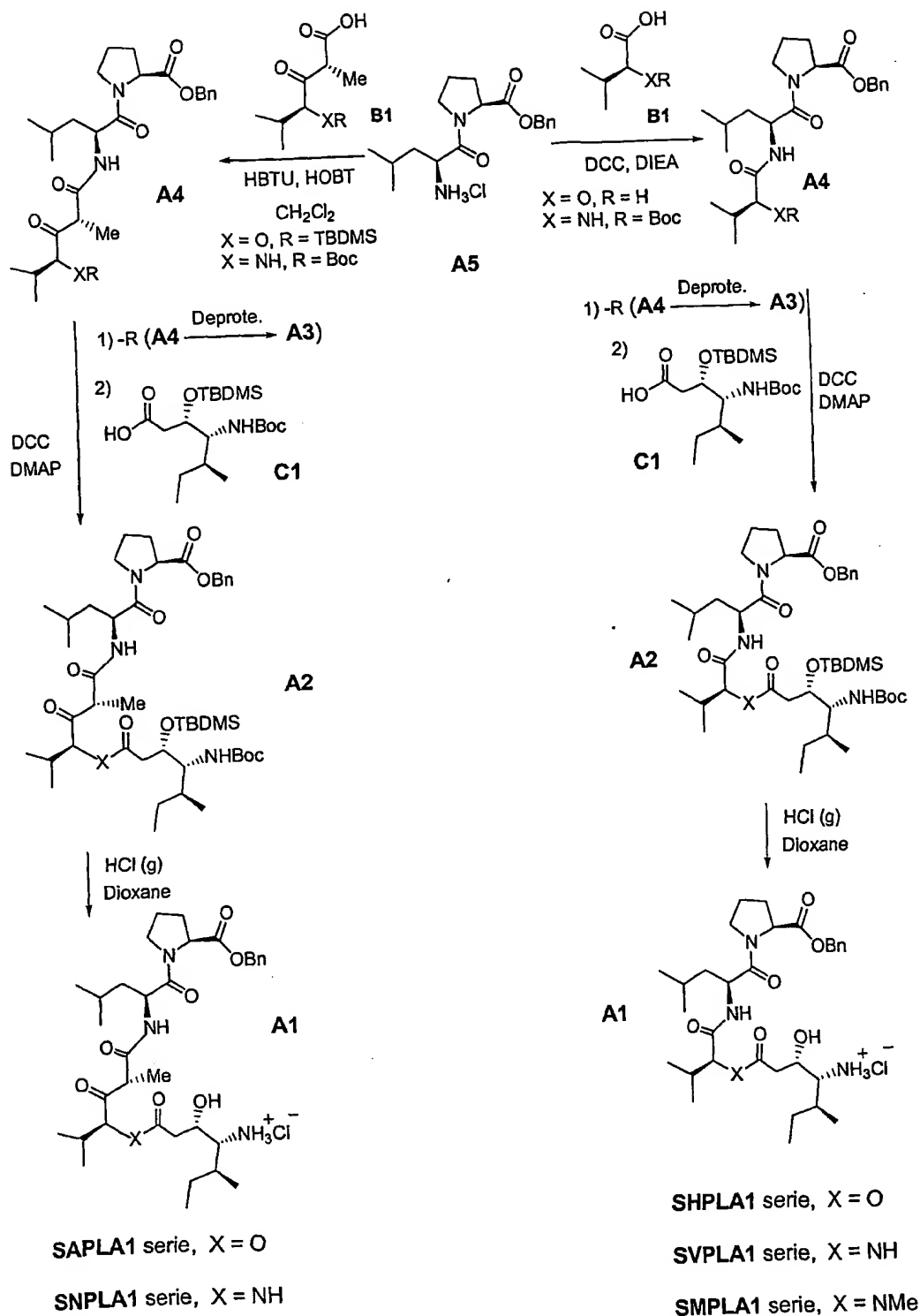
Synthesis of the dipeptide right fragment **D1** has been already described. However the synthesis outlined in Scheme 1 allows the preparation of this intermediate on a kilogram scale. The acid function



of Boc-Thr(OH)-OH was protected with phenacyl bromide to gave directly alcohol **D3**, which was esterified with Z-N(Me)-O(Me)-Tyr-OH using DCC in the presence of DMAP giving **D2**. Removal of the phenacyl group with Zn in acetic acid afforded cleanly fragment **D1**.

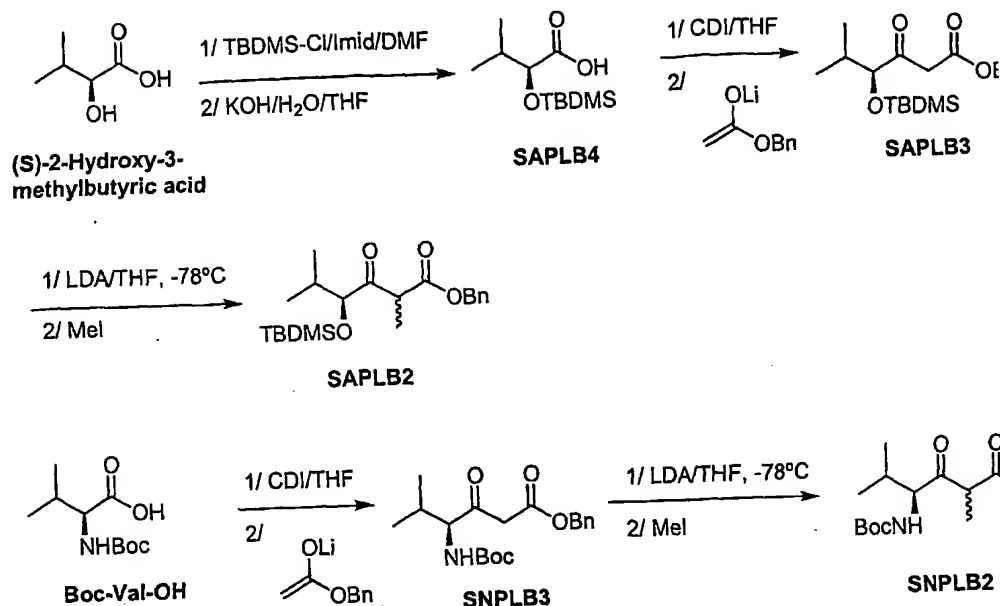
The formation of precursor **A1** is outlined in Scheme 2 for the different series. For the SHPL, SVPL and SMPL series, the first coupling step between Leu-Pro-OBzl (**A5**) and commercially available acid (**B1**) gave directly alcohol **A3** ready for the next reaction with isostatine (**C1**). For the synthesis of SAPL and SNPL series, the route differs to that previously described in that **B1** is a  $\beta$ -ketoester synthesized from  $\alpha$ -( $\alpha'$ -hydroxyiso-valeryl)propionic and  $\alpha$ -( $\alpha'$ -aminoisovaleryl)propionic acid respectively. The synthesis of the fragment **B2** (SAPL and SNPL series) is outlined in scheme 3. Hydrogenolisis from **B2** to **B1** is achieved just before the (one pot) coupling reaction with **A5**.

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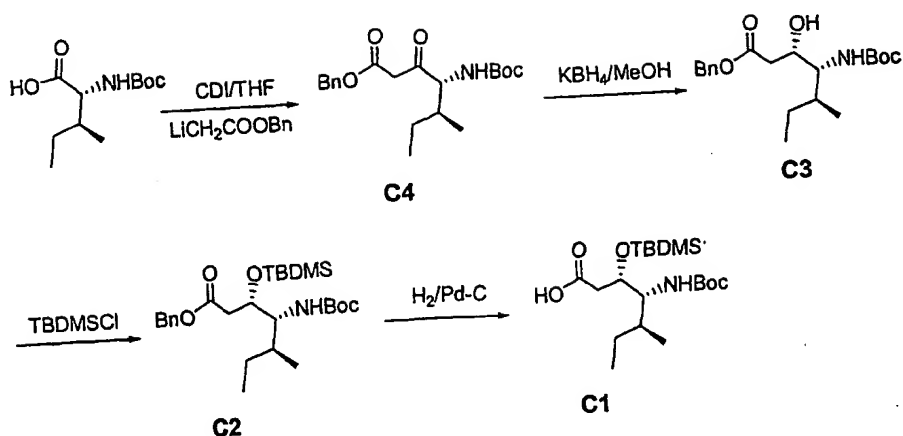
Scheme 2



Scheme 3

The preparation of the entire fragment **A1** rely on the availability of the isostatine portion which has been prepared from the non proteinogenic amino protected Boc-D-alloisoleucine (**C5**) on large quantities. The synthetic route for the preparation of **C1** is outlined in Scheme 4.

Activation of the carboxylic functionality of Boc-D-allo-Ile with carbonyldiimidazol followed by condensation with the lithium enolate of the benzyl acetate, gave the  $\alpha$ -ketoester (**C4**). Stereoselective reduction with KBH<sub>4</sub> in methanol iven **C3**. Protection of the secondary hydroxyl group as the TBDMS ether (**C2**) and hydrogenolysis of the resulting benzyl ester afforded isostatine (**C1**).



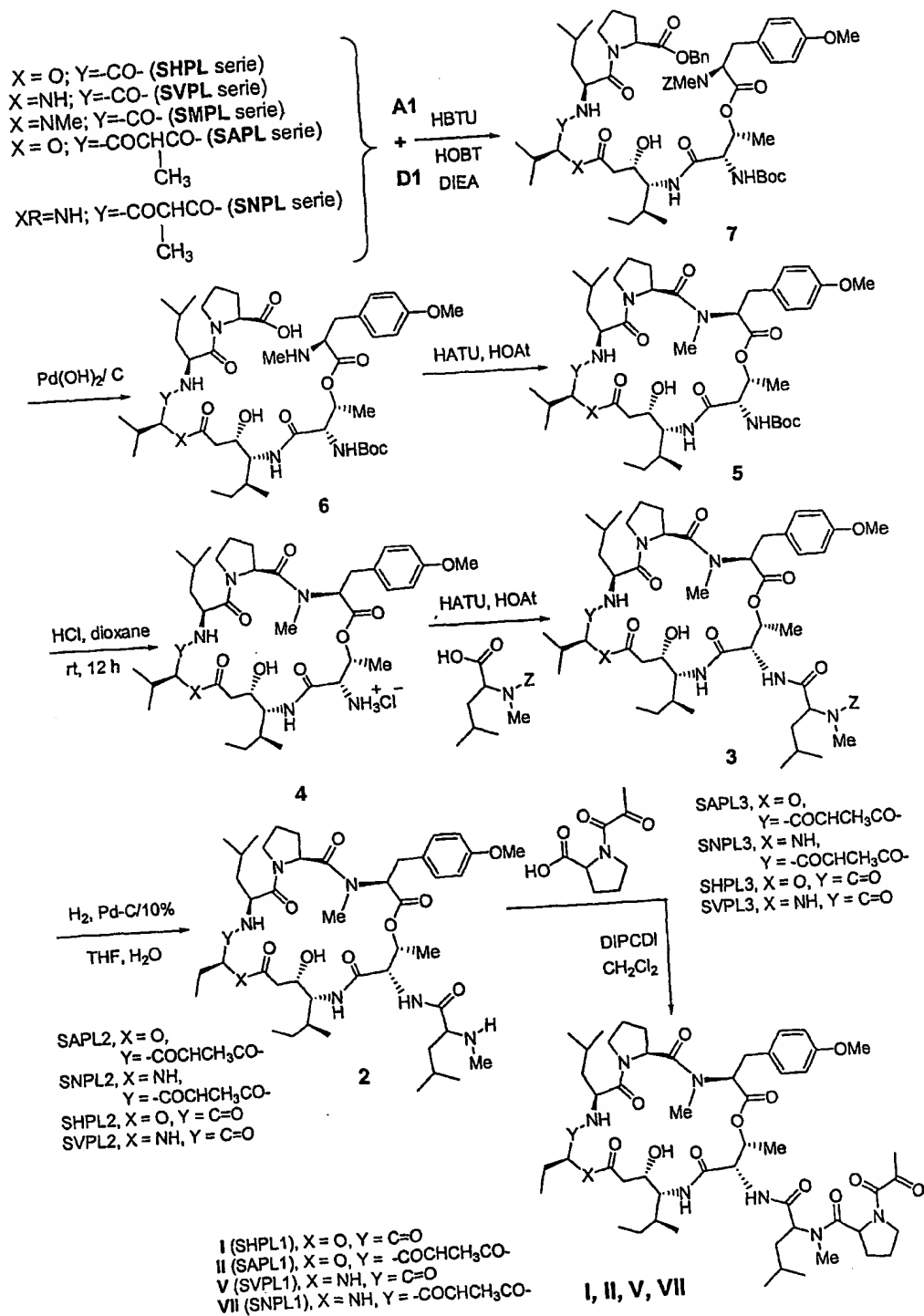
Scheme 4

Subsequent steps yielding compounds **I**, **II**, **V**, and **VII** are depicted in scheme 5.

Coupling of fragments **A1** and **D1** with HBTU/HOBt afforded the linear precursor type **7**. Hydrogenolysis of Cbz and Benzyl protecting groups proceeds out cleanly and smoothly with Pd(OH)<sub>2</sub> to give **6**. Macrocyclization step using HATU/HOAt afforded intermediate **5** in good yield (75%). Hydrogen chloride in dioxane was used to cleave Boc protecting group affording amine **4**. This compound was coupled with Z-NMe-D-Leu-OH to give **3**, which was subjected to hydrogenolysis with Pd-C. The resulting compound **2** was coupled with Pyr-Pro-OH side chain using DIPCDI to afford the corresponding compounds.

Interestingly, precursors of type **2** which are analogs in all aspects mentioned earlier, to didemnin A have served as starting building blocks for the synthesis of some interesting congeners, since the N-terminus, a free secondary amino group, offers a site to attach various acyl groups to the cyclic depsipeptide.

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Scheme 5

In an earlier study (Rinehart et al *J. Med. Chem.* **1996**, 39, 2819-2834) acyl derivatives of didemnin A (dA) **3** were have also found active as the parent compounds **2**. For the aa3 series we found also activity in compounds **3**

Tables 1 and 2 show the IC<sub>50</sub> values found in compounds **1**.

**Table 1: Cytotoxicity of [aa3]-aplidine Congeners IC<sub>50</sub>(Molar)**

Compound/ Serie	P388	A549	HT29	MEL28	DU145
Aplidine (II)/ SAPL1	1,80E-10	1,80E-10	4,50E-10	4,50E-10	
[Hiv] <sup>3</sup> -aplidine (I)/ SHPL1	4,74E-10	4,74E-10	4,74E-10	4,74E-10	
[Val] <sup>3</sup> -aplidine (V)/ SVPL1	1,13E-10	1,13E-10	1,13E-10	1,13E-10	
[Aip] <sup>3</sup> -aplidine (VII)/ SNPL1		9,01E-10	9,01E-10		

Methodology: after Berjeron et al, *Biochem and Bioph Res. Comm.*, **1984**, 121, 3, 848-854.

P388 = Murine lymphoma. A549 = human lung carcinoma. HT-29 = human colon carcinoma. MEL-28 = human melanoma. DU145 = human prostate carcinoma

**Table 2. IC<sub>50</sub> (Molar) values for the Aplidine Family**

Solid Tumors	Line	58	Didemnin B	Aplidine	[Hiv] <sup>3</sup> -
		9LSAPL1	SAPL1	SAPL1	Aplidine
		III	II	I	SHPL1
Bladder	5637	2.50E-08	3.59E-08	9.02E-08	
	MX-1	1.54E-06	1.67E-07	N/A	
Breast	HT-29	8.07E-08	6.87E-07	1.02E-08	
Colon	Hs746t	6.60E-09	2.52E-08	7.16E-08	
Gastric	SK-HEP-	9.21E-08	9.44E-08	2.65E-07	
Liver	1				
NSCL	A549	1.21E-04	2.40E-05	N/A	
	SK-OV-3	1.63E-07	7.20E-08	-	
Ovary	PANC-1	1.52E-10	1.7E-07	-	
Pancreas	FADU	9.79E-08	7.29E-08	3.71E-08	
Pharynx	PC-3	9.00E-08	5.13-07	-	
Prostate	DU-145	-	-	N/A	
Prostate	LNCAP	-	-	1.46E-08	
Prostate	786-O	2.90E-07	8.31E-08	-	
Renal	NCI-	-	-	N/A	
SCL	H187				
Retinoblastoma	Y-79	-	-	-	
	Mel-28	-	-	4.86E-07	
Melanoma	SW 694	3.28E-06	1.49E-06	N/A	
Fibrosarcoma	CHSA	-	-	3.45E-09	
Chondrosarcoma	OSA-FH	-	-	5.89E-09	
Osteosarcoma					

Leukemias/Lymphomas	Line	59		
		Didemnin B	Aplidine	[Hiv] <sup>3</sup> -
		9LSAPL1	SAPL1	Aplidine
		III	II	SHPL1
				I
ALL (Promyelocytic leukemia)	HL-60	1.44E-07	7.89E-08	N/A
ALL (Acute lymphoblastic)	Molt 3	5.45E-07	5.95E-07	1.77E-08
CML (Chronic myelogenous)	K562	3.31E-06	5.72E-07	5.21E-07
ALL (B-cell)	CCRF-SB	6.55E-07	4.72E-07	-
Leukemia (Hairy B-cell)	Mo-B	-	-	-
Leukemia (Plasma cell)	ARH-77	-	1.78E-07	-
Lymphoma (T cell)	H9	2.13E-07	5.25E-07	N/A
Lymphoma (Cutaneous T cell)	Hut 78	3.56E-08	4.47E-08	-
Lymphoma (undifferentiated)	MC116	8.84E-09	9.21E-07	3.82E-07
Lymphoma (Burkitts B cell)	RAMOS	-	-	-
Lymphoma (Histiocytic)	U-937	1.87E-07	5.62E-07	-
Lymphoma (B cell)	MoB	-	-	-
Lymphoma (Burkitts ascites)	P3HR1	5.58E-08	5.34E-08	-
Methodology:		MTT	MTT	MTS (new)

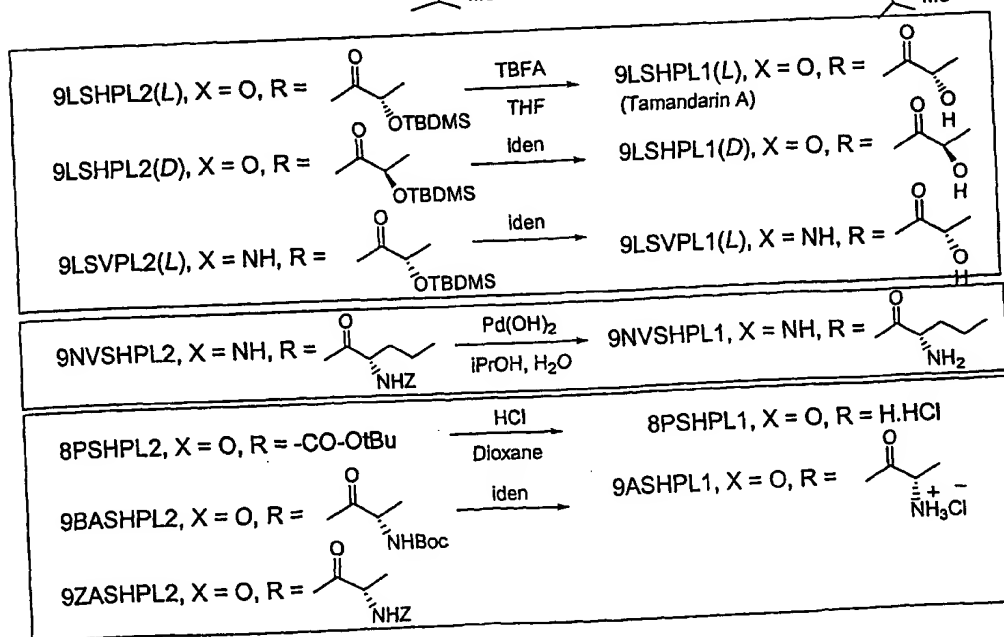
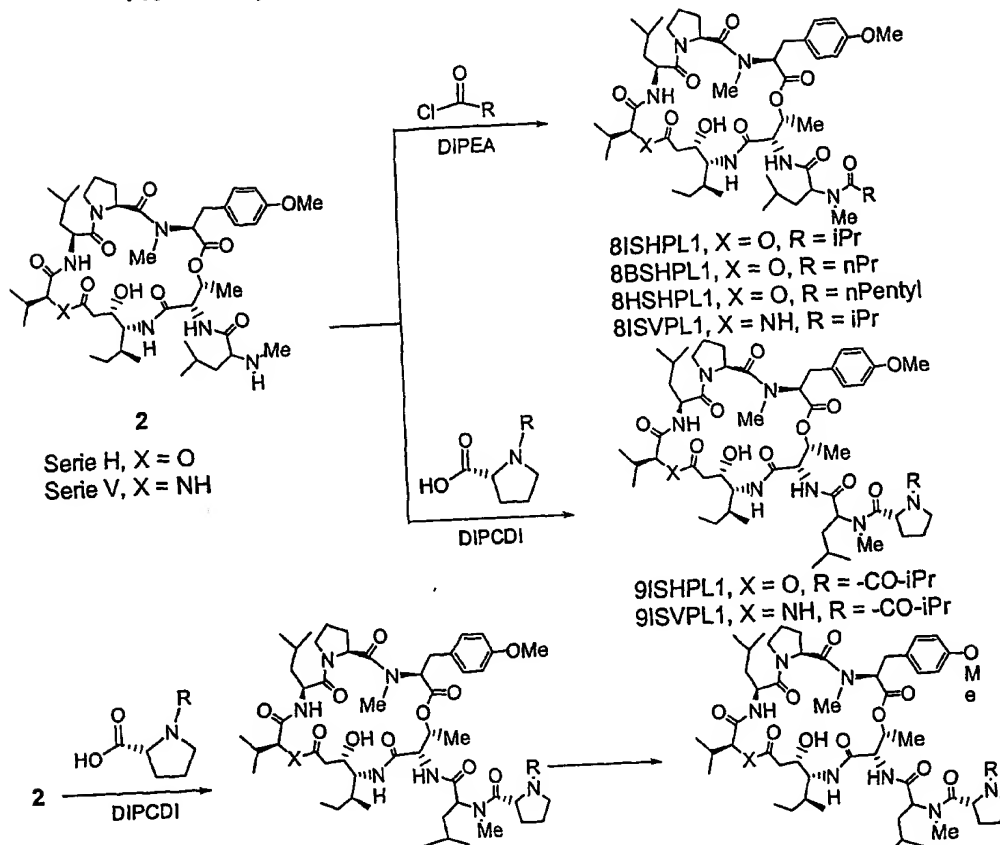
N/A = not active

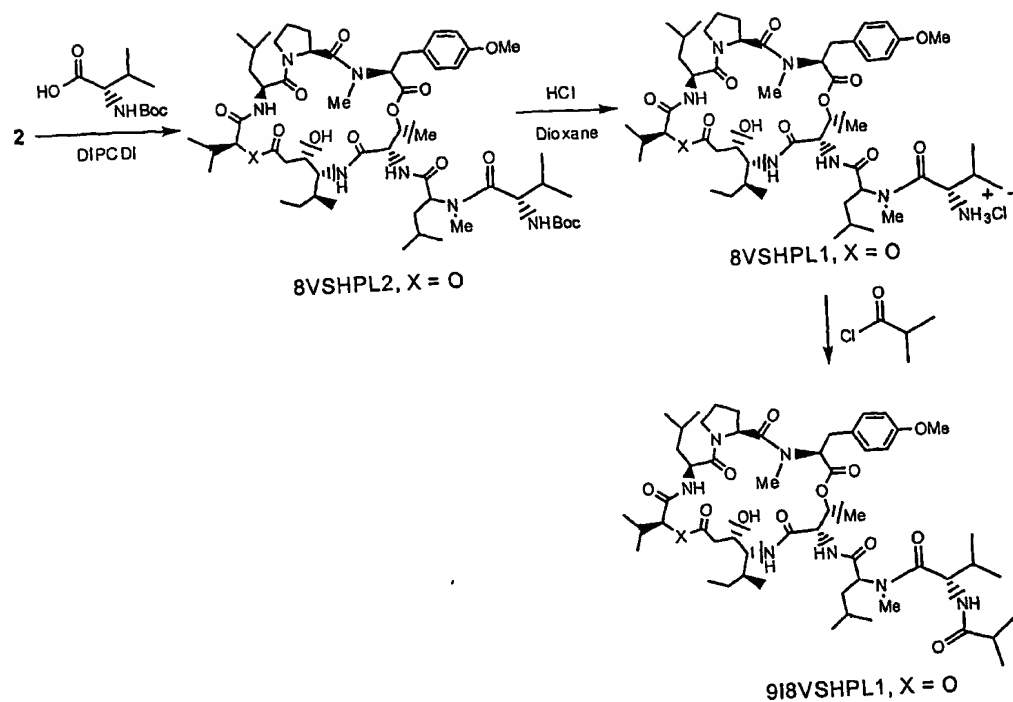


Compounds prepared herein are shown in Schemes 5 and 6. These compounds include N<sup>α</sup>-propionyl-[aa]<sup>3</sup>-dA, N<sup>α</sup>-butyryl-[aa]<sup>3</sup>-dA, N<sup>α</sup>-isobutyryl-[aa]<sup>3</sup>-dA, and N<sup>α</sup>-pentanoyl-[aa]<sup>3</sup>-dA.

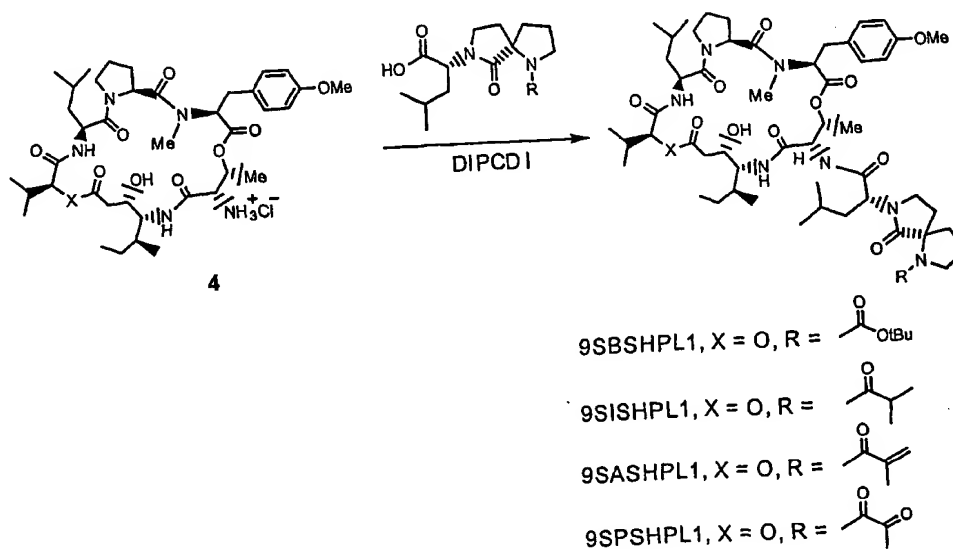
Analogues which have two acyl subunits after the N-terminus of the aa<sup>3</sup>-dA core were prepared to examine the structural factors contributing to the specificity to certain tumors. The diacyl compounds isobutyryl-Pro-OH, O-isobutyryl-Lac-Pro-OH, N-Benzyl-Ala-Pro-OH were prepared and condensed with type 2 compounds by the DIPCDI method to obtain respectively, after deprotection and purification, [aa]<sup>-3</sup>[isobutyryl]<sup>9</sup>-aplidine, [O-isobutyryl-Lac]<sup>9</sup>-aplidine, [Ala]<sup>9</sup>-aplidine.

## Preferred Aplidine derivatives: Compounds from Series: H, V



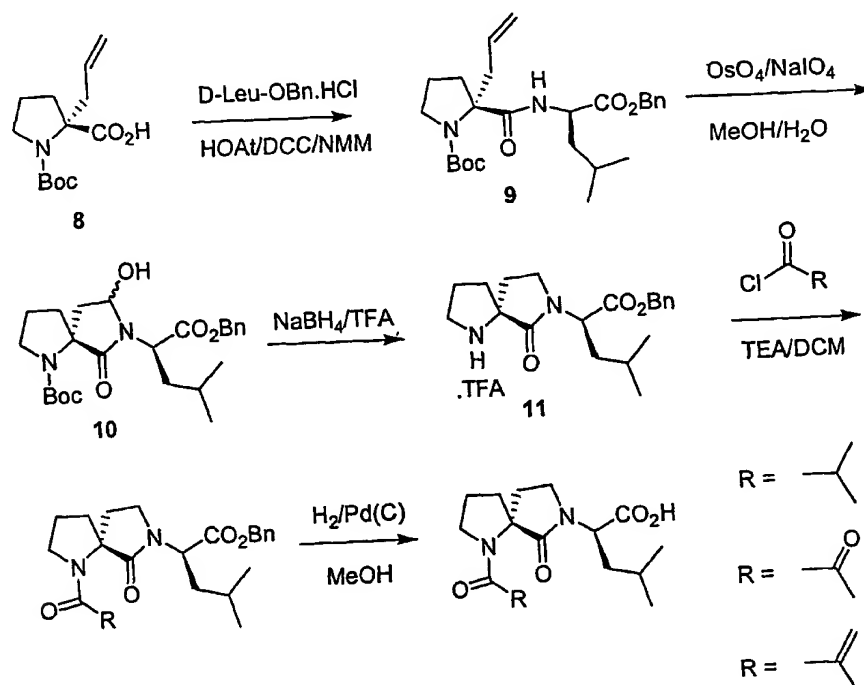


Spirocompounds were also linked to form active compounds:

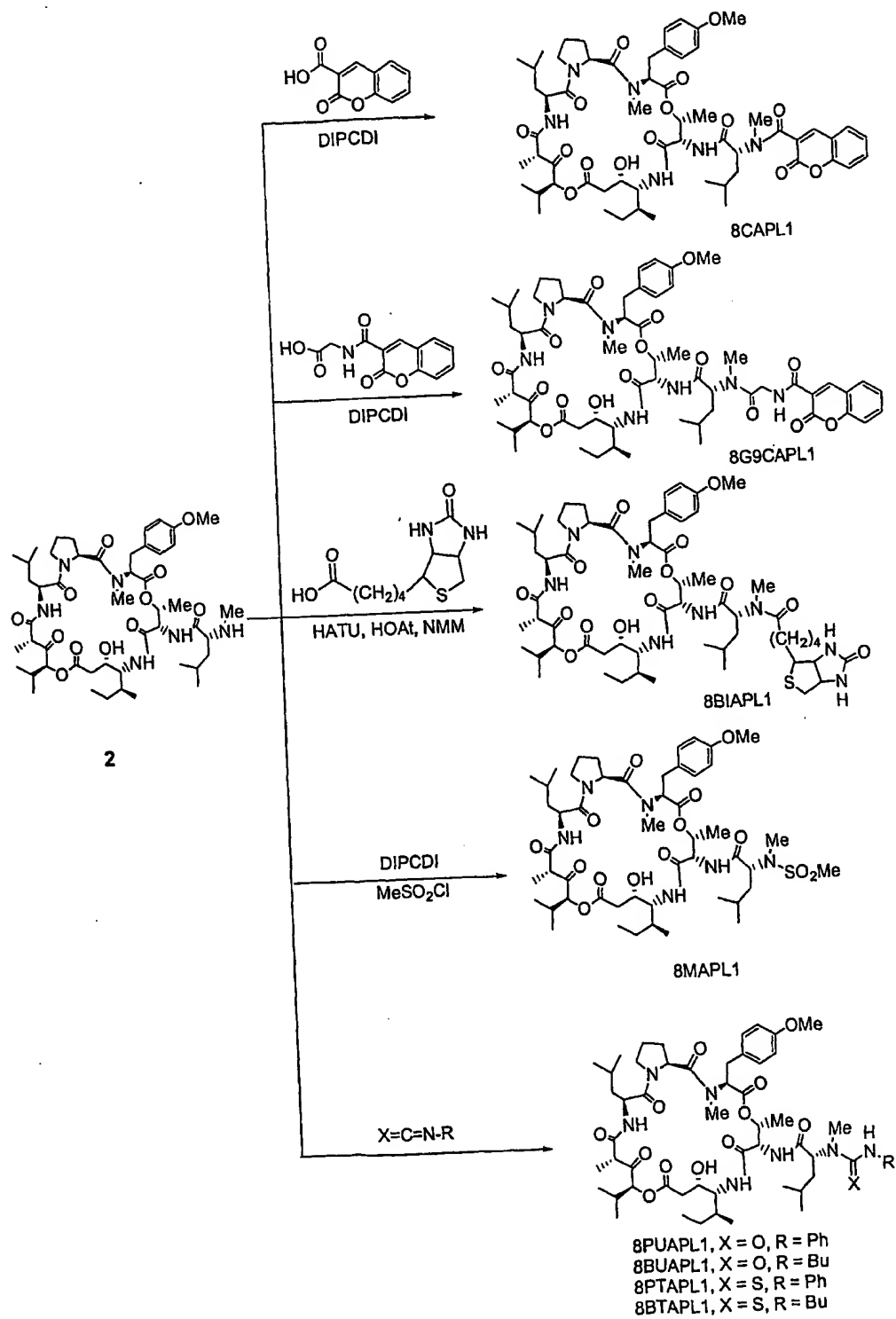


Scheme 6

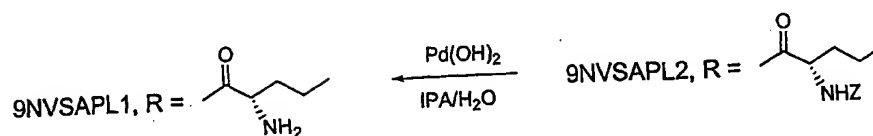
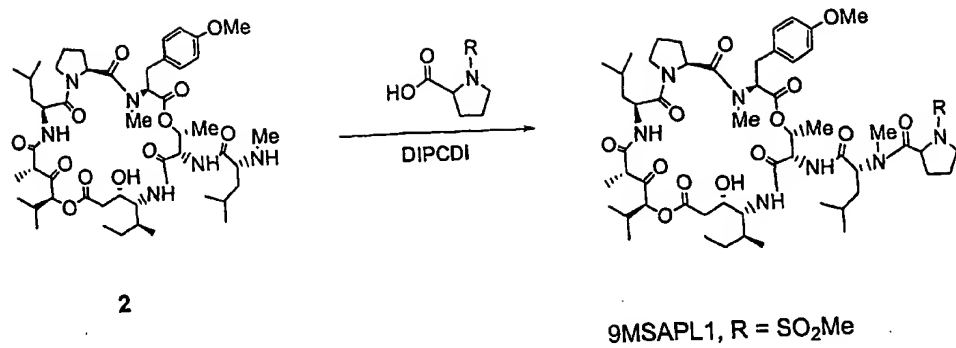
The spirocyclic fragments synthesis is outlined in scheme 7. The synthesis started from the previously reported compound **8**. Ref: a) Seebach, D. *et al. J. Am. Chem. Soc.* **1983**, *105*, 5390-5398. b) Genin, M. J. *et al. J. Org. Chem.* **1993**, *58*, 2334-2337.



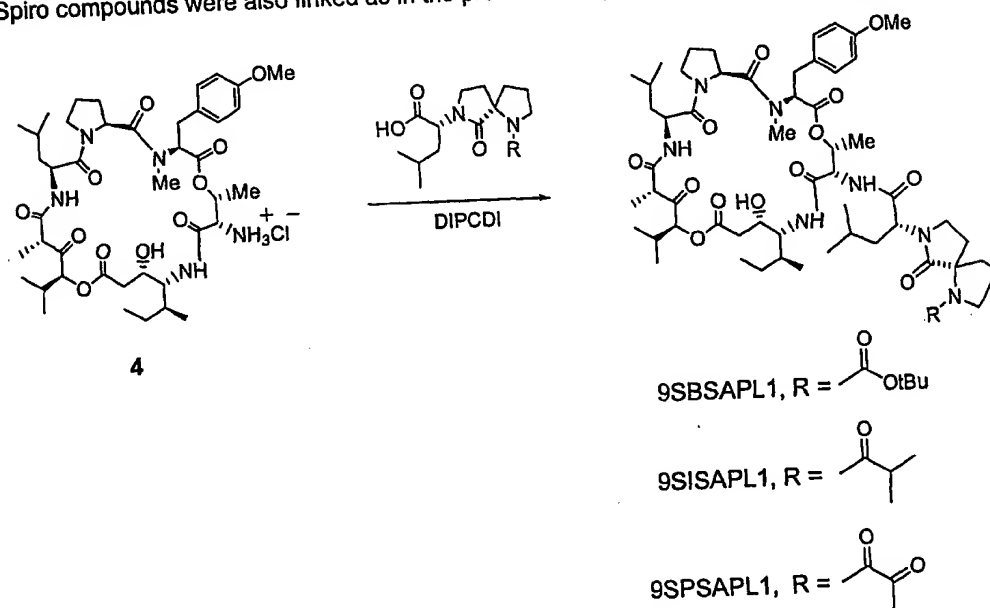
Scheme 7



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Spiro compounds were also linked as in the previous series to give active compounds:



Scheme 8

Table 3: Cytotoxicity of aplidine derivatives IC<sub>50</sub> (Molar)

Serie	Description	MolW	P388	A549
SNPL3	3-[Aip]-Z-didemnin A	1076		9,29E-11
8PUSAPL1	8-[Phenylurea]-didemnin A	1062		9,42E-11
8BUSAPL1	8-[Butylurea]-didemnin A	1042		9,59E-11
8ISVPL1	3-[val]-8-[isobutyryl]- aplidine	956		1,05E-10
9NVSAPL1	9-[norvaline]-aplidine	1139		4,39E-10
9ISHPL1	3-[Hiv]-9-[Isobutyryl]- aplidine	1054		4,74E-10
9ISVPL1	3-[Val]-9-[Isobutyryl]- aplidine	1053		4,75E-10
8ISHPL1	3-[hiv]-8-[isobutyryl]- didemnin A	957		5,22E-10
9ASHPL1	3-[Hiv]-9-[Ala]- aplidine	1091		9,16E-10
8PSHPL2	3-[Hiv]-8-[Boc-Pro]- didemnin A	1084		9,22E-10
9NVSHPL1	3-[Hiv]-9-[Nva]- aplidine	1083		9,23E-10
8PTSAPL1	8-[Phenylthiourea]-didemnin A	1078		9,27E-10
8CSAPL1	8-[Coumarin]-didemnin A	1062		9,41E-10
8BTSAPL1	8-[Butylthiourea]-didemnin A	1058		9,45E-10
9LSHPL1(L) A)	3-[Hiv]-9-[L-Lac]- aplidine (Tamandarine A)	1056		9,47E-10
9LSHPL1(D)	3-[Hiv]-9-[D-Lac]- aplidine	1056		9,47E-10
9LSVPL1(L)	3-[Val]-9-[Lac]- aplidine	1055		9,48E-10
8MSAPL1	8-[Methylsulphonyl]-didemnin A	1021		9,79E-10
SVPL3	3-[val]-Z-didemnin A	1020	9,8E-10	9,8E-10
8PSHPL1	3-[Hiv]-8-[Pro]- didemnin A	984		1,01E-09
8VSHPL1	3-[Hiv]-8-[Val]- didemnin A	986		1,01E-09
8BSHPL1	3-[Hiv]-8-[butyryl]- aplidine	957		1,04E-09
SVPL2	3-[val]-didemnin A	886	1,1E-09	1,13E-09
SHPL2	3-[Hiv]-didemnin A	886	1,1E-09	1,13E-09
SAPL3	Z-Didemnin A	1077	9,3E-09	2,32E-09
9NVSAPL2	9-[Z-Nva]-aplidine	1273	7,9E-09	3,93E-09
9ZASHPL2	3-[Hiv]-9-[Z-ala]- aplidine	1189		4,21E-09
SAPL2	Didemnin A	943	5,30E-09	4,24E-09
8G9CSAPL1	8-[Gly]-9-[Coumarin]- didemnin A	1172		4,26E-09
8BISAPL1	8-[Biotin]-didemnin A	1169		4,27E-09
9BSHPL1	3-[Hiv]-7,8-[Spiro]-9-[Boc]- aplidine	1096		4,56E-09
SHPL3	3-[Hiv]-Z-didemnin A	1021	4,9E-09	4,9E-09

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9NVSHPL2	3-[Hiv]-9-[Z-Nva]- aplidine	1217	8,22E-09
9SPSAPL1	7,8-[Spiro]-9-[pyr]-aplidine	1122	8,55E-09
9LSHPL2(L)	3-[Hiv]-9-[lac(OTBDMS)]- aplidine	1170	8,55E-09
9BASHPL2	3-[Hiv]-9-[Boc-Ala]- aplidine	1155	8,65E-09
9SBSAPL1	7,8-[Spiro]-9-[Boc]-aplidine	1152	8,68E-09
8VSHPL2	3-[Hiv]-8-[Boc-Val]- aplidine	1086	9,21E-09
8V9ISHPL1	8-[Val]-9-[Isobutiryl]- didemnin A	1056	9,46E-09
8HSHPL1	3-[Hiv]-8-[hexanoyl]- didemnin A	985	1,01E-08
9LSVPL2(L)	3-[Val]-9-[Lac(OTBDMS)]- aplidine	1169	1,02E-08
SNPL2	3-[Aip]-didemnin A	942	1,06E-08
9LSHPL2(D)	3-[Hiv]-9-[D-Lac(OTBDMS)]- aplidine	1170	8,55E-08
9SISAPL1	7,8-[Spiro]-9-[Isobutyryl]-aplidine	1122	8,91E-08
9SPSHPL1	3-[Hiv]-7,8-[Spiro]-9-[Pyr]- aplidine	1066	9,38E-08
9SISHPL1	3-[Hiv]-7,8-[Spiro]-9-[Isobutyryl]- aplidine	1066	9,38E-08
9SASHPL1	3-[Hiv]-7,8-[Spiro]-9-[Acriloyl]- aplidine	1064	9,39E-08

Serie	Description	HT29	MEL28	DU145
		9,29E-		
SNPL3	3-[Aip]-Z-didemnin A	11		
		9,42E-		
8PUSAPL1	8-[Phenylurea]-didemnin A	11		
		9,59E-		
8BUSAPL1	8-[Butylurea]-didemnin A	11		
		1,05E-		
8ISVPL1	3-[val]-8-[isobutyryl]- aplidine	10		
		4,39E-		
9NVSAPL1	9-[norvaline]-aplidine	10		
		4,74E-		
9ISHPL1	3-[Hiv]-9-[Isobutyryl]- aplidine	10		
		4,75E-		
9ISVPL1	3-[Val]-9-[Isobutyryl]- aplidine	10		
		5,22E-		
8ISHPL1	3-[hiv]-8-[isobutyryl]- didemnin A	10		
		9,16E-		
9ASHPL1	3-[Hiv]-9-[Ala]- aplidine	10		



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		9,22E-		
		10		
8PSHPL2	3-[Hiv]-8-[Boc-Pro]- didemnin A	9,23E-		
		10		
9NVSHPL1	3-[Hiv]-9-[Nva]- aplidine	9,27E-		
		10		
8PTSAPL1	8-[Phenylthiourea]-didemnin A	9,41E-		
		10		
8CSAPL1	8-[Coumarin]-didemnin A	9,45E-		
		10		
8BTSAPL1	8-[Butylthiourea]-didemnin A	9,47E-		
	3-[Hiv]-9-[L-Lac]- aplidine	10		
9LSHPL1(L)	(Tamandarine A)	9,47E-		
		10		
9LSHPL1(D)	3-[Hiv]-9-[D-Lac]- aplidine	9,48E-		
		10		
9LSVPL1(L)	3-[Val]-9-[Lac]- aplidine	9,79E-		
		10		
8MSAPL1	8-[Methylsulphonyl]-didemnin A	9,8E-10	9,8E-10	9,8E-10
SVPL3	3-[val]-Z-didemnin A	1,01E-		
		09		
8PSHPL1	3-[Hiv]-8-[Pro]- didemnin A	1,01E-		
		09		
8VSHPL1	3-[Hiv]-8-[Val]- didemnin A	1,04E-		
		09		
8BSHPL1	3-[Hiv]-8-[butyryl]- aplidine	1,13E-		
		09		
SVPL2	3-[val]-didemnin A	1,13E-09	1,13E-09	
		09		
SHPL2	3-[Hiv]-didemnin A	1,13E-09		
		4,64E-		
SAPL3	Z-Didemnin A	09	4,64E-09	
		3,93E-		
9NVSAPL2	9-[Z-Nva]-aplidine	09	3,93E-09	3,93E-09
		4,21E-		
9ZASHPL2	3-[Hiv]-9-[Z-ala]- aplidine	09		
		8,48E-		
SAPL2	Didemnin A	09	1,13E-09	

		4,26E-		
		09		
8G9CSAPL1	8-[Gly]-9-[Coumarin]- didemnin A	4,27E-		
		09		
8BISAPL1	8-[Biotin]-didemnin A	4,56E-		
		09		
9SBSHPL1	3-[Hiv]-7,8-[Spiro]-9-[Boc]- aplidine	4,9E-09	4,9E-09	4,9E-09
SHPL3	3-[Hiv]-Z-didemnin A	8,22E-		
		09		
9NVSHPL2	3-[Hiv]-9-[Z-Nva]- aplidine	8,9E-09		
9SPSAPL1	7,8-[Spiro]-9-[pyr]-aplidine	8,55E-		
		09		
9LSHPL2(L)	3-[Hiv]-9-[lac(OTBDMS)]- aplidine	8,65E-		
		09		
9BASHPL2	3-[Hiv]-9-[Boc-Ala]- aplidine	8,68E-		
		09		
9SBSAPL1	7,8-[Spiro]-9-[Boc]-aplidine	9,21E-		
		09		
8VSHPL2	3-[Hiv]-8-[Boc-Val]- aplidine	9,46E-		
		09		
8V9ISHPL1	8-[Val]-9-[Isobutiryl]- didemnin A	1,01E-		
		08		
8HSHPL1	3-[Hiv]-8-[hexanoyl]- didemnin A	8,55E-		
		09		
9LSVPL2(L)	3-[Val]-9-[Lac(OTBDMS)]- aplidine	1,06E-		
		08		
SNPL2	3-[Aip]-didemnin A	8,55E-		
		08		
9LSHPL2(D)	3-[Hiv]-9-[D-Lac(OTBDMS)]- aplidine	8,91E-		
		08		
9SISAPL1	7,8-[Spiro]-9-[Isobutyryl]-aplidine	9,38E-		
		08		
9SPSHPL1	3-[Hiv]-7,8-[Spiro]-9-[Pyr]- aplidine	9,38E-		
	3-[Hiv]-7,8-[Spiro]-9-[Isobutyryl]-	08		
9SISHPL1	aplidine	9,39E-		
		08		
9SASHPL1	3-[Hiv]-7,8-[Spiro]-9-[Acirloyl]- aplidine			

Spiro = [(5R)-1-(substituent at 9)-7-[(1R)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane]<sup>7-9</sup>

(1) This compound has been described in the literature: *Joc Org. Chem.* 2000, 65, 782-792. Their synthesis was published before their discovery

(i): *Org. Lett.*, 2000, vol 0, No. 0, A-D

Methodology: after Berjeron et al, *Biochem and Bioph Res. Comm.*, **1984**, 121, 3, 848-854

388 = Murine lymphoma. A549 = human lung carcinoma. HT-29 = human colon carcinoma. MEL-28 = human melanoma. DU145 = human prostate carcinoma

### List of Abbreviations

#### *Miscellaneous*

AA	Amino acid
Ist	Isostatine
Hip	Hydroxyisovalerylpropionic acid
Hiv	Hydroxyisovaleric acid
Aip	Aminoisovalerylpropionic acid
Lac	Lactic acid
LC	Liquid Chromatography
HPLC	High Performance Liquid Chromatography
TLC	Thin Layer Chromatography
M.p.	Melting point
R <sub>t</sub>	Retention time
Quant.	Quantitative yield
ESI-MS	Electrospray Ionization Mass Spectra

#### *Protecting groups*

Bn	Benzyl
----	--------

Boc	<i>tert</i> -Butyloxycarbonyl
TBDMS	<i>tert</i> -Butyldimethylsilyl
Z	Benzyloxycarbonyl
Pac	Phenyl acetic

*Solvents*

THF	Tetrahydrofuran
Hex	Hexane
ACN	Acetonitrile
DCM	Dichlorometane
EtOAc	Ethyl acetate
DMF	Dimethylformamide
MTBE	Methyl <i>tert</i> butyl ether
Et <sub>2</sub> O	Diethyl ether
<i>t</i> -BuOH	<i>tert</i> -Butanol
TFA	Trifluoroacetic acid
MeOH	Methanol
EtOH	Ethanol
IPA	Isopropanol

*Reagents*

CDI	1,1'-Carbonyldiimidazole
HOBt	1-Hydroxybenzotriazole
HBTU	N-[(1H-Benzotriazol-1-yl)(dimethylamino)methylene]-N-methanaminium hexafluorophosphate N-oxide
BOP-Cl	Bis(2-oxo-3-oxazolidinyl)phosphinic chloride
HATU	N-[(dimethylamino)-1H-1,2,3,-triazolo[4,5-b]pyridin-1-ylmethylene]-N-methyl-methanaminium hexafluorophosphate N-oxide
HOAt	1-Hydroxy-7-aza-benzotriazole
DCC	Dicyclohexylcarbodiimide

DIPCDI	N,N'-Diisopropylcarbodiimide
TBAF	Tetrabutylammonium fluoride
AcOH	Acetic acid
p-TsOH	<i>p</i> -toluensulphonic acid
DMAP	4-Dimethylamino pyridine
NMM	N-Methyl morpholine
DIPEA	Diisopropylethylamine
TEA	Triethylamine
TFA	Trifluoroacetic acid

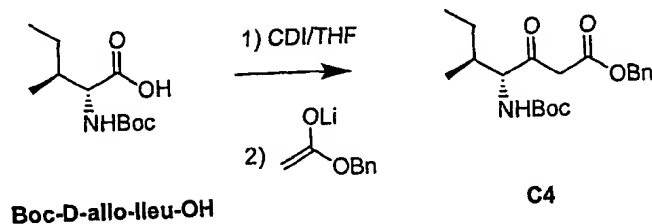
## General Procedure

All manipulations were conducted under an inert atmosphere of argon. All solvents were reagent grade (used in work-ups) or HPLC grade (used as reaction and or as purification solvent). Anhydrous solvents were used directly as supplied by the manufacturer. Tetrahydrofuran was freshly distilled prior to use to remove stabilizer. All other reagents were commercial compounds of the highest purity available. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel aluminium sheets (60, F254) precoated with a fluorescent indicator. Visualization was effected using ultraviolet light (254 nm), phosphomolybdic acid (5% w/v) in 95% ethanol, or vainilline. Proton and carbon magnetic resonance spectra ( $^1\text{H}$ ,  $^{13}\text{C}$ -NMR) were recorded on a Varian-300 (300 MHz) Fourier transform spectrometer, and chemical shifts were expressed in parts per million (ppm) relative to  $\text{CHCl}_3$  as an internal reference (7.26 ppm for  $^1\text{H}$  and 77.0 for  $^{13}\text{C}$ ). Multiplicities are designated as singlet (s), doublet (d), doublet of doublets (dd), doublet of triplets (dt), triplet (t), quartet (q) multiplet (m), and broad singlet (bs), and coupling constants (J) were expressed in Hz. Optical rotations (in degrees) were measured with a Jasco P1020 polarimeter. Electrospray ionization mass spectra (ESI-MS) were obtained on a Hewlett Packard Series 1100 MSD. Elemental. Flash column chromatography was carried out on E. Merck silica gel 60 (240-400 mesh) or RP C18 (40-63  $\mu\text{m}$ ) using the solvent systems listed under individual experiments.

The following procedures describe the synthesis of intermediates obtained toward aplidine (SAPL), [Aiv]<sup>3</sup>-aplidine (SNPL), [Hiv]<sup>3</sup>-aplidine (SHPL), [Val]<sup>3</sup>-aplidine (SVPL), and [MeVal]<sup>3</sup>-aplidine (SMPL).

### Example 1

**Synthesis of Benzyl (4R, 5S)-4-(tert-Butoxycarbonylamino)-5-Methyl-3-oxoheptanoate (C4)**

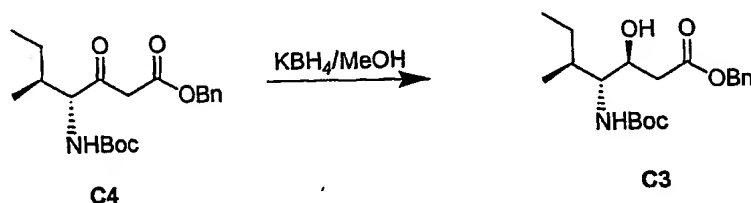


To a solution of Boc-D-allo-leu-OH (15.26 g, 65.9 mmol) in dry THF (200 ml) at 0° C under argon, was added CDI (16.04 g, 98.96 mmol). After 15 min, the mixture was allowed to warm to room temperature, and stirred over a period of 16 h. The resulting solution was cooled to -78°C, and added via cannula to a well stirred solution of benzyl lithium enolate cooled at -78° C (625 ml, 0.37 M), [prepared by adding dropwise a solution of benzyl acetate (33.34 ml), in THF (165 ml) to a solution of lithium diisopropylamide (0.36M) in THF/hex 3:1 (642 ml) at -78°C]. The temperature should be kept < -75 ° C. The reaction mixture was stirred at -78°C for 60 min. Then, it was allowed to come to -10° C (30 min), recooled to -78°C and quenched with saturated aq. ammonium chloride (200 ml), then extracted with DCM (3x500 ml) at room temperature. The combined extracts were washed successively with aq sat. NaHCO<sub>3</sub> (500 ml) and brine (200 ml). Drying (Na<sub>2</sub>SO<sub>4</sub>) followed by removal of solvent gave an oil, which was coated on silica C18 and loaded to the top of a LC-RPC18 [Lichroprep RPC-18 (40-60 microns) column. Elution using a gradient ACN-H<sub>2</sub>O (60 to 100% ACN)] yielded the product **C4** as a colourless oil (16.7 g, 70%).  $[\alpha]^{20}_D$  -20.0 (c 1, CHCl<sub>3</sub>), TLC: R<sub>f</sub> = 0.32 (Merck, RP-C18, ACN-H<sub>2</sub>O 7:3).

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$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.77 (d, 3H), 0.94 (t, 3H), 1.25 (s, 9H), 1.60 (m, 1H), 1.90 (m, 2H), 3.58 (s, 2H), 4.47 (dd, 1H), 5.00 (d, 1H), 5.18 (s, 2H), 7.35 (bs, 5H).

## Example 2

**Synthesis of (3S, 4R, 5S)-N-(tert-Butoxycarbonyl)isostatine benzyl ester (C3)**

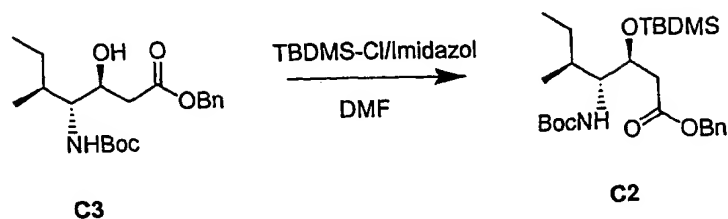
**C4** (16.7 g, 45.9 mmol) was dissolved in methanol (690 ml) at 0°C. Potassium Borohydride (7.43 g, 137.8 mmol) was added to the stirred solution and after 30 min the reaction was quenched with aq HCl (0.1 N) to pH 4, and extracted with DCM (300 ml). The extract was washed successively with aq  $\text{NaHCO}_3$  (100 ml, sat) and brine (100 ml). Drying ( $\text{Na}_2\text{SO}_4$ ) followed by removal of solvent afforded alcohol **C3** (15.7 g, 93%) as a colourless oil.  $R_f$  = 0.45 (hex-EtOAc 2:1);  $[\alpha]_D^{25}$  = -9.5 (c 0.76,  $\text{CHCl}_3$ );  $R_f$  = 0.45 (EtOAc-Hex 1:2).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.81 (d, 3H), 0.90 (t, 3H), 1.20 (m, 1H), 1.36 (m, 1H), 1.40 (s, 9H), 1.90 (m, 1H), 2.55 (dd, 1H), 2.70 (dd, 1H), 3.20 (d, 1H), 3.61 (m, 1H), 3.90 (m, 1H), 4.40 (d, 1H), 5.18 (s, 2H), 7.40 (bs, 5H).

## Example 3



### Synthesis of Boc-(3S, 4R, 5S)-Ist(TBDMS)-OBn (C2)



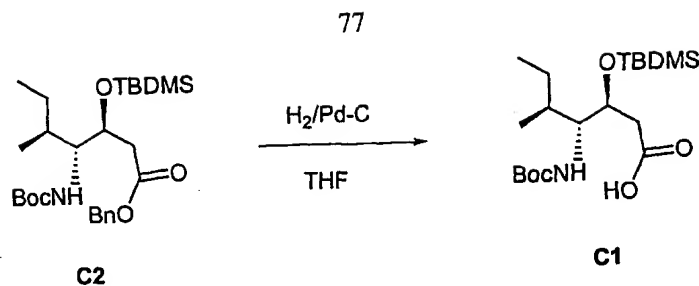
To a solution of **C3** (15.7 g, 42.9 mmol) in dry DMF (65 ml) at 0° C, imidazol (8.77 g, 128.8 mmol), DMAP (1.57 g, 12.81 mmol), and TBDMS-Cl (19.42 g, 128.8 mmol) were added. The reaction mixture was allowed to warm to room temperature overnight, then it was partitioned between Et<sub>2</sub>O (200 ml) and successively with aq HCl (100 ml, 0.1 N), aq NaHCO<sub>3</sub> (100 ml, sat) and brine (50 ml). After drying (Na<sub>2</sub>SO<sub>4</sub>) and solvent removal, the residue was purified by flash LC (silica gel, hex) to yield **C2** (19.96 g, 97%).

$[\alpha]_D = 10.6$  (c 1.01,  $\text{CHCl}_3$ );  $R_f = 0.73$  (EtOAc-Hex 1:2).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.15 (s, 6H), 0.82 (s, 9H), 0.85 (d, 3H), 0.89 (t, 3H), 1.18 (m, 1H), 1.35 (m, 1H), 1.41 (s, 9H), 1.77 (m, 1H), 2.45 (dd, 1H), 2.60 (dd, 1H), 3.62 (m, 1H), 4.20 (m, 1H), 4.40 (d, 1H), 5.05 (d, 1H), 5.15 (d, 1H), 7.40 (bs, 5H).

### Example 4

### Synthesis of Boc-(3S, 4R, 5S)-Ist(TBDMS)-OH (C1)

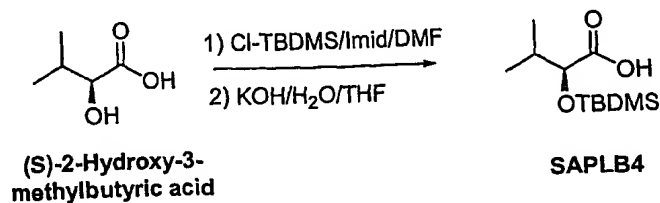


To a solution of **C2** (10.48 g, 21.8 mmol) in THF (110 ml), degassed and purged with argon, was added Pd/C 10% (2.096 g, 20% by weight). The mixture was stirred under H<sub>2</sub> (1 atm) for 16 h, then filtered over a 0.45 mm teflon filter and concentrated at reduced pressure to give 7.8 g of a colorless oil. Colorless crystals (6 g, 70%) were obtained after crystallization in ACN at -20° C.  $[\alpha]_D^{20}$  1.8 (c 0.594, DCM) [Lit.  $[\alpha]_D$  1.74 (c 2.64, CHCl<sub>3</sub>). *Synthesis*, **1991**, 294]; R<sub>f</sub> = 0.45 (Merck HPTLC, RP-C18, ACN-H<sub>2</sub>O 8:2).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.18 (s, 6H), 0.82 (s, 9H), 0.85 (d, 3H), 0.89 (t, 3H), 1.10-1.20 (m, 2H), 1.42 (s, 9H), 1.80 (m, 1H), 2.50 (m, 2H), 3.58 (m, 1H), 4.11 (m, 1H).

#### Example 5

#### Synthesis of (2S)-2-tert-Butyl(dimethyl)silyloxy-3-methylbutyric acid (SAPLB4)



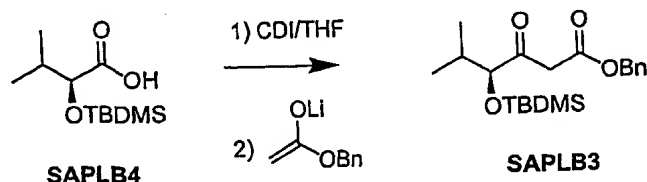
To a stirred solution of (S)-2-hydroxy-3-methylbutyric acid (21.12 g, 181.9 mmol) in DMF (91 ml) at 0°C under argon were added Imidazol

(27.24 g, 400.11 mmol) and DMAP (6.94 g, 54.56 mmol). After 5 min, *tert*-Butyldimethylchlorosilane (60.31 g, 400.11 mmol) was added. The mixture was allowed to warm to 23°C and stirred overnight. The crude reaction mixture was partitioned between Et<sub>2</sub>O (250 ml) and aq HCl (250 ml, 0.1N). The organic phase was washed successively with aq. NaHCO<sub>3</sub> (250 ml, sat), and brine (250 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated at reduced pressure to afford the bissilylated product as a pale yellow oil. A solution of this product in THF (100 ml) was added dropwise (10 min) to a cooled (0°C) solution of KOH (30.47g, 543 mmol) in THF/H<sub>2</sub>O (543 ml: 181 ml). After 40 min the reaction mixture was partitioned between H<sub>2</sub>O (300 ml) and Et<sub>2</sub>O (500 ml). The aqueous phase was partitioned between cold (0°C) aq HCl (200 ml, 3N) and EtOAc (5 x 250 ml). The combined organic extracts were dried (Na<sub>2</sub>SO<sub>4</sub>) filtered and concentrated under reduced pressure to afford **SAPLB4** as a pale yellow oil (38.38 g, 91%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.02 (m, 6H), 0.90 (m, 15H), 2.08 (m, 1H), 4.06 (d, 1H).

### Example 6

### Synthesis of Benzyl (4S)-4-tert-Butyl(dimethyl)silyloxy-5-Methyl-3-oxohexanoate (SAPLB3)



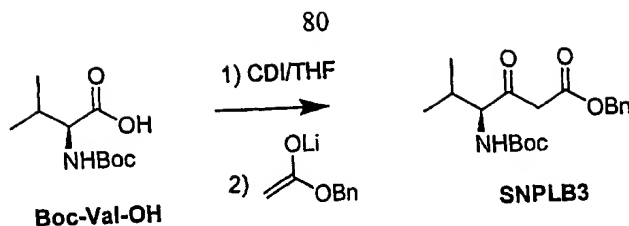
To a solution of (S)-2-(tertButyldimethylsilyloxy)-3-methylbutyric acid (15.31 g, 65.9 mmol) in dry THF (200 ml) at 0° C under argon, was

added CDI (16.04 g, 98.96 mmol). After 15 min, the mixture was allowed to warm to room temperature, and stirred over a period of 16 h. The resulting solution was cooled to  $-78^{\circ}\text{C}$ , and added via cannula to a well stirred solution cooled at  $-78^{\circ}\text{C}$  of benzyl lithium enolate (625 ml, 0.37 M), [prepared by adding dropwise a solution of benzyl acetate (33.34 ml), in THF (165 ml) to a solution of lithium diisopropylamide (0.36M) in THF/hex 3:1 (642 ml) at  $-78^{\circ}\text{C}$ ]. The temperature should be kept  $< -75^{\circ}\text{C}$ . The reaction mixture was stirred at  $-78^{\circ}\text{C}$  for 60 min. Then, it was allowed to come to  $-10^{\circ}\text{C}$  (30 min), re-cooled to  $-78^{\circ}\text{C}$  and quenched with aq. ammonium chloride (200 ml, sat), then extracted with DCM (3x500 ml) at room temperature. The combined extracts were washed successively with aq  $\text{NaHCO}_3$  (500 ml, sat) and brine (200 ml). Drying ( $\text{Na}_2\text{SO}_4$ ) followed by removal of solvent gave an oil, which was coated on silica C18 and loaded to the top of a LC-RPC18 [Lichroprep RPC-18 (40-60 microns), column. Elution using a grad. ACN- $\text{H}_2\text{O}$  (60 to 100% ACN)] yielded **SAPLB3** as a colourless oil (16.1 g, 70%).  $[\alpha]_D -25$  (c 0.5, MeOH);  $R_f = 0.32$  (Merck, RP-C18, ACN- $\text{H}_2\text{O}$  7:3).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.02 (m, 6H), 0.92 (m, 15H), 1.92 (m, 1H), 3.63 (s, 2H), 3.80 (s, 2H), 3.38 (d, 1H), 5.17 (d, 1H), 5.20 (d, 1H), 7.35 (bs, 5H).

#### Example 7

#### Synthesis of Benzyl (4S)-4-tert-Butoxycarbonylamino-5-Methyl-3-oxohexanoate (SNPLB3)



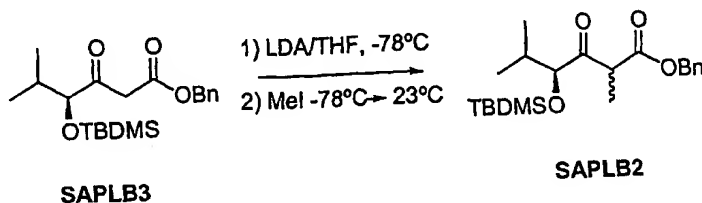
Following the procedure obtained for the synthesis of **SAPLB3** from Boc-Val-OH (10 g, 46.0 mmol), the title compound was obtained after purification by flash LC (silica gel, gradient hex-EtOAc 10:1 to 5:1) as an oil (6.9 g, 43%).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.77 (d,  $J=7$ , 3H), 0.98 (d,  $J=7$ , 3H), 1.44 (s, 9H), 2.22 (m, 1H), 3.58 (s, 2H), 4.31 (m, 1H), 5.03 (m, 1H), 5.18 (s, 2H), 7.34 (bs, 5H).

ESI-MS Calcd for  $\text{C}_{19}\text{H}_{27}\text{NO}_5$ : 349.19. Found (m/z): 372.1 ( $\text{M}+\text{Na}$ ) $^+$ .

#### Example 8

**Synthesis of Benzyl (2RS,4S)-4-tert-butyl(dimethyl)silyloxy-2,5-dimethyl-3-oxohexanoate (SAPLB2).**



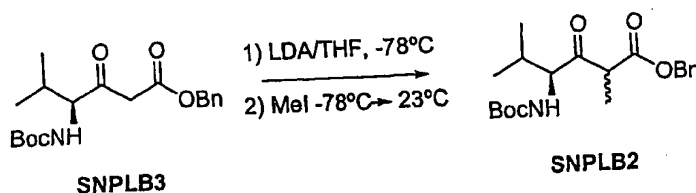
The ester **SAPLB3** (15.12 g, 41.49 mmol) in dry THF (43 ml) was added dropwise to a solution of lithium diisopropylamine at  $-78^\circ\text{C}$  [prepared by adding butyllithium (1.6 M solution in hex; 31.12 ml, 49.79 mmol) dropwise to diisopropylamine (7.26 ml, 51.86 mmol) in dry THF (83 ml) under Ar at  $-78^\circ\text{C}$  for 30 min.] The mixture was stirred for 0.5 h

and then, Iodomethane was added (52.11 ml, 829.8 mmol). The mixture was allowed to warm to 23° C and then, stirring continues for 24 h. Additional Iodomethane was added (2.67 ml, 42 mmol) and the mixture was stirred 24 h further or until disappearance of starting material. The mixture was then partitioned between aq. NH<sub>4</sub>Cl (50 ml, sat) and EtOAc (2x 200 ml). The organic layer was washed successively with aq. NaHCO<sub>3</sub> (100 ml, sat), brine (100 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to give a yellow oil (12 g). Pure product (**SAPLB2**) was obtained after purification by LC-silica gradient hex-Et<sub>2</sub>O 100:0 to 100:2 as a diastereomeric mixture of epimers at C2 (10 g, 63%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.01 (s, 3H), 0.02 (s, 3H), 0.91 (m, 15H), 1.30 (d, 3H), 2.01 (m, H), 4.01 (m, 1H), 5.10 (d, 1H), 5.15 (d, 1H), 7.34 (bs, 5H).

#### Example 9

**Synthesis of Benzyl (2RS,4S)-4-tert-butoxycarbonylamino-2,5-dimethyl-3-oxohexanoate (SNPLB2).**



Following the procedure obtained for the synthesis of **SAPLB2**, starting from **SNPLB3** (10 g, 46.0 mmol), the title compound was obtained after purification by flash LC (silica gel, gradient hex-EtOAc 10:1 to 5:1) as a diastereomeric mixture (1:1) of epimers at C2 (4.4 g, 62%). R<sub>f</sub> = 0.4 and 0.37 (silica, Hex/EtOAc 3:1).

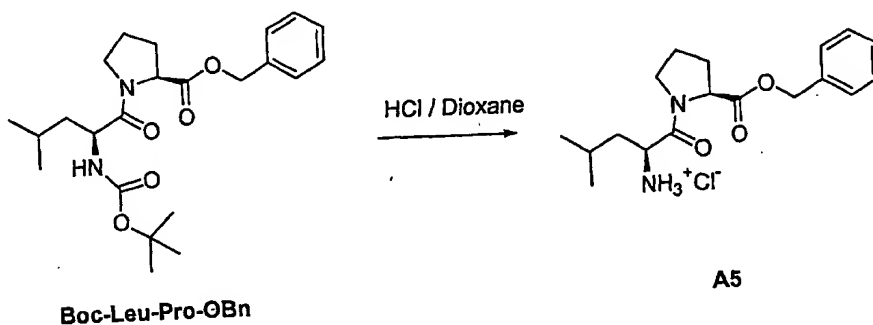
82

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.72 (m, 3H), 0.86 (m, 3H), 1.37 (m, 3H), 1.44 (s, 9H), 2.22 (m, 1H), 3.79 (m, 1H), 4.43 (m, 1H), 5.02 (m, 1H), 5.16 (m, 2H), 7.34 (m, 5H).

ESI-MS Calcd for  $\text{C}_{20}\text{H}_{29}\text{NO}_5$ : 363.20. Found (m/z): 364.1 ( $\text{M}+\text{H}$ ) $^+$ .

### Example 10

#### Synthesis of Leu-Pro-OBn as chlorhydrate salt (A5)



To a flask containing Boc-Leu-Pro-OBn (113.8 g, 272 mmol) a solution of hydrogen chloride in dioxane (209 ml, 5.3 N) was added and the stirring was continued for 5 h or until total conversion by TLC (disappearance of starting material:  $R_f$  = 0.47 (hex-EtOAc 2:1, silica). The solution was concentrated under reduced pressure and the resulting oil was chased with  $\text{CHCl}_3$  (3x50 ml),  $\text{CHCl}_3$ -MTBE (30 ml-50 ml), MTBE (50 ml) and hex (50 ml). The residue was dried under vacuum (16 h) to remove residual HCl, to give the title compound as a white solid. **A5** (96.4 g, 100%) was used directly without further purification in the next step.  $[\alpha]_D^{22}$  -85.21 ( $c$  = 1,  $\text{CHCl}_3$ ).

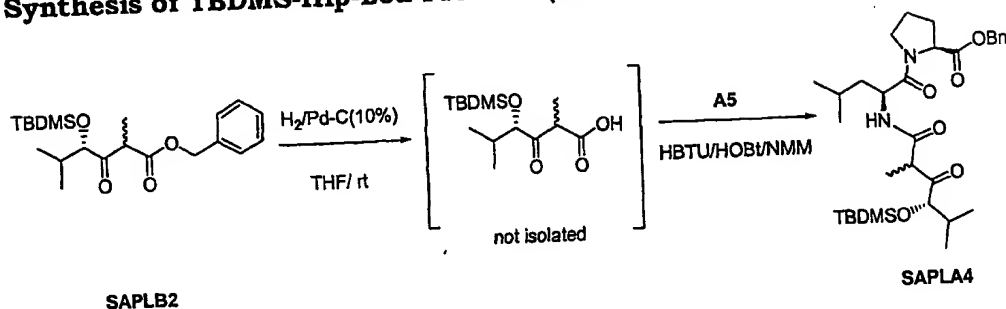
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.92 (d,  $J$ =7.1, 3H), 0.96 (d,  $J$ =7.1, 3H), 1.55 (m, 1H), 1.82-2.14 (m, 5H), 2.26 (m, 1H), 3.42 (m, 1H), 3.90 (m,

1H), 4.32 (bs, 1H), 4.64 (m, 1H), 5.01 (d, J=11.5, 1H), 5.16 (d, J=11.5, 1H), 7.34 (m, 5H), 8.40 (bs, 3H).

ESI-MS Calcd for  $C_{18}H_{26}N_2O_3$ : 318.19. Found (m/z): 319.2 (M+H)<sup>+</sup>.

### Example 11

#### Synthesis of TBDMS-Hip-Leu-Pro-OBn (SAPLA4)



To a flask containing a degassed solution of **SAPLB2** (20g, 52.88 mmol) in THF anh. (158 ml), provided with gas inlet-outlet tubes, was added 10% Pd/C (6.0 g, 30% by wt.) under Ar. Then, a stream of hydrogen is passed through for 8h or until complete conversion by TLC (disappearance of starting material). The resulting mixture was bubbled with Ar to displace hydrogen, and filtered under Ar in a sintered glass funnel through a sort pad of celite, to a cooled flask (-5° C) containing HOBt (7.17 g, 52.88 mmol) and HBTU (21.0 g, 55.53 mmol). Additional THF (158 ml) was added to wash the celite. To the mixture (at -5° C) were added NMM (5.8 ml, 52.88 mmol) and after 5 min a cooled (-5° C) solution containing: **A5** (31.96 g, 89.81 mmol), NMM (16 ml, 145 mmol) and DMF (120 ml), fresh prepared. The reaction mixture was allowed to warm to rt and stirred for 14 h. The crude reaction was filtered and the solvent removed under reduced pressure. To the residual solution of DMF, EtOAc (300 ml) was added and washed successively with aq HCl



(200 ml, 0.1 N), aq.  $\text{NaHCO}_3$  (200 ml, sat.) and rinsed with brine (300 ml). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ) filtered and concentrated. The resulting material was coated with silica (EtOAc as solvent), and chromatographed on silica gel eluting with a gradient EtOAc:hex 1:5 to 1:1 to yield **SAPLA4** (26.8 g, 78%) as a thick colourless oil. This product is a 1:1 mixture of diastereomers.  $R_f = 0.5$  (silica, Hex/EtOAc 1:1, dark blue/vainillin).

IR (film, DCM) 3295, 3060 and 3040, 2957, 2934, 2880, 2858, 1736, 1634, 1528, 1454, 1387, 1252, 1171, 1070  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.01 (s, 3H), 0.03 (s, 3H), 0.85-0.97 (m, 12H), 0.92 (s, 9H), 0.93 (s, 9H), 1.33 (d,  $J = 7.0$ , 3H), 1.37 (d,  $J = 7.0$ , 3H), 2.40-2.65 (m, 3H), 1.92-2.28 (m, 4H), 3.64-3.76 m, 1H), 4.69-4.82 (m, 1H), 5.05 (d,  $J = 11.8$ , 1H), 5.20 (d,  $J = 11.8$ , 2H), 6.73 (d,  $J = 8.9$ , 1H), 6.98 (d,  $J = 9.0$ , 1H), 7.34 (bs, 5H).

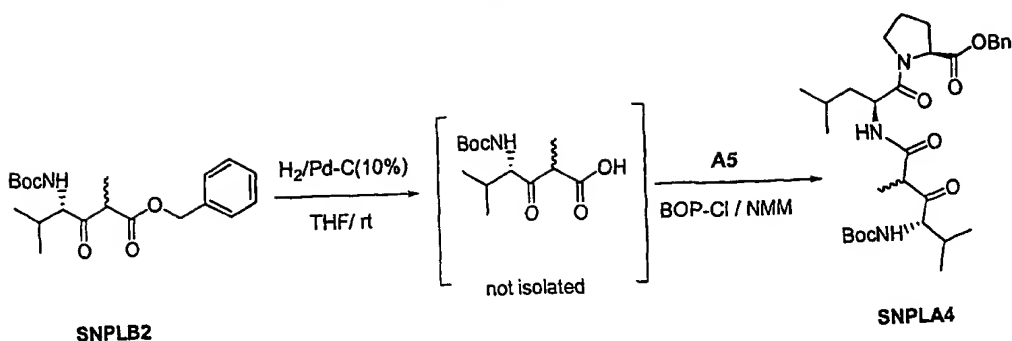
$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.24, -4.81, 15.70, 17.43, 17.57, 18.84, 21.48, 21.61, 18.05, 23.28, 24.43, 24.55, 24.76, 25.68, 28.87, 31.36, 31.77, 41.27, 41.67, 48.68, 48.55, 48.89, 58.71, 66.84, 83.84, 83.29, 128.09, 128.47, 135.40, 169.24, 170.67, 170.89, 171.16, 171.20, 209.11, 211.62.

$m/z$  (FAB) 611.5  $[(M + \text{Na})^+, 15]$ , 589.5  $[(M + \text{H})^+, 100]$ ;  $m/z$  (FABHRMS) 589.369 045,  $\text{C}_{32}\text{H}_{52}\text{N}_2\text{O}_6\text{Si}$  requires  $(M+\text{H})^+$ , 589.367 291

#### Example 12

#### Synthesis of Boc-Aip-Leu-Pro-OBn (SNPLA4)

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To a degassed solution of **SNPLB2** (2.3 g, 6.32 mmol) in dry THF (30 ml) was added 10% Pd/C (0.74 g, 16% by wt.) and then hydrogenated at atmospheric pressure for 5h. 30 min or until complete conversion by TLC (disappearance of starting material). The resulting mixture was filtered through a sort pad of celite and additional THF (20 ml) was added to wash the celite. To the filtered solution (at  $-5^{\circ}\text{C}$ ) were added BOP-Cl (1.77 g, 6.96 mmol) and NMM (765  $\mu\text{l}$ , 6.91 mmol) and after 30 min a cooled ( $-5^{\circ}\text{C}$ ) solution containing: **A5** (3.15 g, 8.85 mmol), NMM (1.88 ml, 8.84 mmol) and DMF (14 ml) prepared 10 min before. The reaction mixture was allowed to warm to rt and stirred for 17 h. The crude reaction was filtered and the solid washed with EtOAc (100 ml). The combined organic solutions was successively washed with aq  $\text{KHSO}_4$  (50ml, 10%), aq.  $\text{NaHCO}_3$  (50 ml, sat.) and brine (50 ml). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ) filtered and concentrated in vacuo, and the resulting material was chromatographed on silica gel eluting with a gradient EtOAc:hex 1:4 to 1:1 to yield **SNPLA4** (750 mg, 20 %) as a white solid. This product is a mixture of diastereomers.  $R_f = 0.26$  and  $0.17$  (silica, Hex/EtOAc 1:1).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.74-1.04 (m, 12H), 1.31-1.70 (m, 6H), 1.43 (s, 9H), 2.01 (m, 3H), 2.22 (m, 2H), 3.60 (m, 2H), 3.77 (m, 1H), 4.40

86

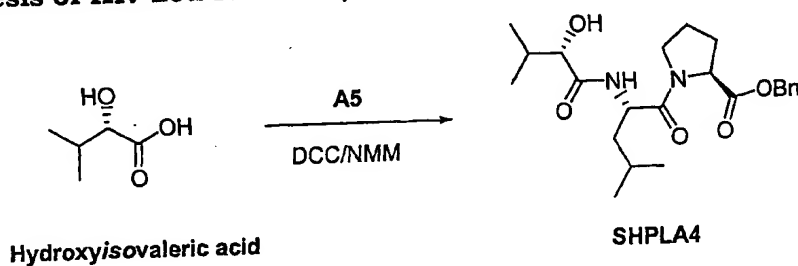
(m, 1H), 4.58 (m, 1H), 4.69 (m, 1H), 5.14 (m, 2H), 6.75 (d,  $J = 8.7$ , 1H), 7.04 (d,  $J = 7.8$ , 1H), 7.34 (bs, 5H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  16.74, 16.91, 20.22, 21.94, 23.53, 24.80, 25.06, 28.48, 29.14, 41.29, 41.41, 46.98, 49.45, 49.64, 51.26, 59.06, 63.70, 64.27, 67.08, 79.86, 80.10, 128.32, 128.48, 128.74, 135.74, 156.28, 169.15, 169.32, 171.18, 171.91.

ESI-MS Calcd for  $\text{C}_{31}\text{H}_{47}\text{N}_3\text{O}_7$ : 573.34. Found (m/z): 574.4  $[(\text{M}+\text{H})]^+$ .

### Example 13

#### Synthesis of Hiv-Leu-Pro-OBn (SHPLA4)



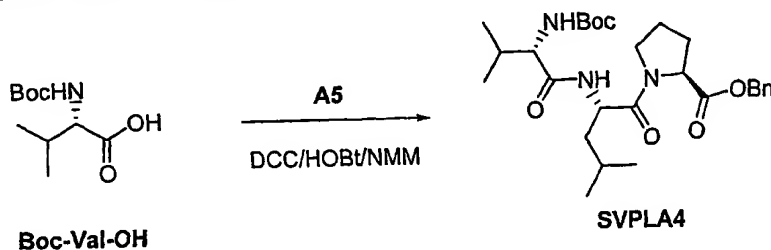
To a flask containing **A5** (2.06g, 4.78 mmol) in DCM (5 ml) at 0°C, NMM (506 mg, 5.01 mmol) was added with stirring. After 15 min (2S)-2-hydroxy-3-methylbutanoic acid (hydroxyisovaleric acid) (487 mg, 4.78 mmol) and DCC (986 mg, 4.78 mmol) were added in portions. The reaction mixture was allowed to warm to 23°C and stirred for 14 h. The suspension was diluted with  $\text{CHCl}_3$  (25 ml) and partitioned between aq HCl (10 ml, 1N), aq  $\text{NaHCO}_3$  (10 ml, sat) and brine (10 ml) dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The residue was purified by flash LC (silica gel, gradient hex-EtOAc 1:1 to 1:3) to give **SHPLA4** (1.13 g, 80%) as a white solid.  $R_f = 0.46$  (hex-EtOAc 1:2).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.81, (d,  $J = 7.0$ , 3H) 0.92 (m, 6H), 0.97 (d,  $J = 7.0$ , 3H), 1.42 (m, 1H), 1.63 (m, 2H), 2.00 (m, 3H), 2.19 (m, 2H), 3.60 (m, 1H), 3.85 (m, 1H), 3.88 (d,  $J = 4.8$ , 1H), 4.46 (m, 1H), 4.80 (m, 1H), 5.06 (d,  $J = 12.3$ , 1H), 5.14 (d,  $J = 12.3$ , 1H), 7.32 (m, 5H), 7.41 (d,  $J = 8.4$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  15.32, 19.08, 21.38, 23.11, 24.41, 24.69, 28.77, 31.31, 40.54, 46.81, 48.20, 58.82, 66.69, 75.76, 127.93, 128.14, 128.37, 135.28, 171.39, 171.95, 173.94.

#### Example 14

##### Synthesis of Boc-Val-Leu-Pro-OBn (SVPLA4)



To a flask containing Boc-Valine-OH (652 mg, 3 mmol) in DCM (6 ml) at  $0^\circ\text{C}$  was added NMM (0.35 ml, 3.15 mmol). After stirring for 15 min, **A5** (1.065 g, 3 mmol), HOBt (405 mg, 3.0 mmol) and DCC (650 mg, 3.15 mmol) were added in portions. The reaction mixture was allowed to warm to  $23^\circ\text{C}$  and stirred for 14 h. The suspension was diluted with DCM (25 ml) and washed successively with aq  $\text{KHSO}_4$  (2x10ml, 10%), aq  $\text{NaHCO}_3$  (2x10 ml, sat) and brine (10 ml) dried over  $\text{Na}_2\text{SO}_4$  filtered and concentrated under reduced pressure. The residue was purified by flash LC (silica, gradient hex-EtOAc 2:1 to 1:1) to give **SVPLA4** (1.48 g, 93%) as a white solid.  $R_f = 0.57$  (hex-EtOAc 1:2).

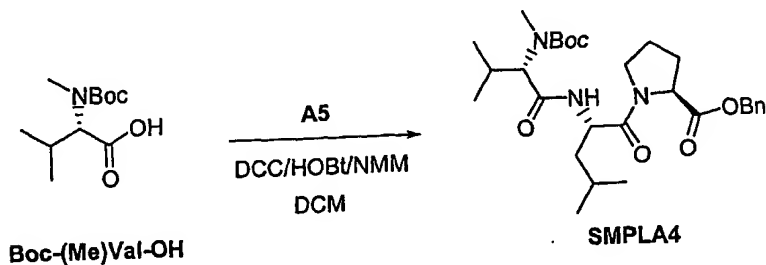
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.83-0.95 (m, 12H), 1.2-1.4 (m, 1H), 1.42 (s, 9H), 1.40-1.51 (m, 1H), 1.60-1.75 (m, 1H), 1.82-2.20 (m, 5H), 3.50-3.60 (m, 1H), 3.74-3.78 (m, 1H), 3.91 3.96 (m, 1H), 4.52-4.57 (s, 1H), 4.75-4.77 (m, 1H), 5.04 (bs, 1H)-, 5.05 (d,  $J=12.3$ , 1H), 5.17 (d,  $J=12.3$ , 1H), 6.60 (d,  $J = 8.4$ , 1H), 7.26-7.35 (m, 5H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  7.61, 19.19, 21.66, 23.23, 24.43, 24.78, 24.87, 25.55, 28.20, 28.87, 30.91, 33.86, 41.68, 46.73, 48.86, 58.77, 59.74, 66.82, 79.66, 128.08, 128.21, 128.46, 135.47, 155.66, 170.83, 171.33, 171.60.

ESI-MS Calcd for  $\text{C}_{28}\text{H}_{43}\text{N}_3\text{O}_6$ : 517.32. Found (m/z): 518.2  $[(\text{M}+\text{H})]^+$ .

#### Example 15

##### Synthesis of Boc-Me-Val-Leu-Pro-OBn (SMPLA4)



Following the procedure described for the synthesis of **SVPLA4**, starting from **A5** (1.07 mg, 3.00 mmol) and Boc-(Me)Val-OH (694 mg, 3.00 mmol), the title compound was obtained as a white solid (1.39 g, 87%) after purification by flash LC (silica gel, gradient hex-EtOAc 2:1 to 1:1).  $R_f = 0.51$  (Hex-EtOAc 1:2).

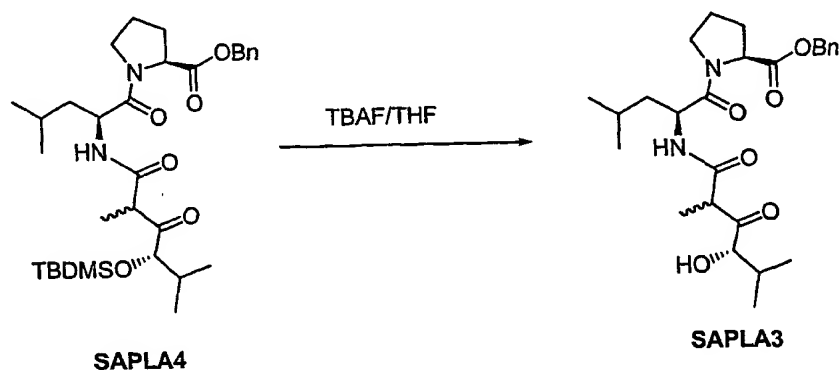
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.83-0.91 (m, 12H), 1.45 (s, 9H), 1.93-2.03 (m, 4H), 2.18-2.22 (m, 2H), 2.76 (s, 3H), 3.40-3.50 (m, 2H) 3.55-3.62 (m, 1H), 3.75-3.85 (m, 1H), 4.00-4.10 (m, 1H), 4.50-4.60 (m, 1H), 4.70-4.82 (m, 1H), 5.07 (d,  $J=11.2$ , 1H), 5.24 (d,  $J=11.2$ , 1H), 6.20 (m, 0.5H), 6.50 (m, 0.5 H), 7.26-7.35 (m, 5H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  18.43, 19.77, 21.53, 23.23, 24.47, 24.77, 25.89, 25.30, 28.85, 41.25, 46.67, 48.51, 58.75, 64.05, 66.79, 128.06, 128.18, 128.44, 135.49, 170.12, 170.80, 171.66

ESI-MS Calcd for  $\text{C}_{29}\text{H}_{45}\text{N}_3\text{O}_6$ : 531.33. Found ( $m/z$ ): 532.3 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 16

##### Synthesis of Hip-Leu-Pro-OBn (SAPLA3)



To a flask containing **SAPLA4** (15.86 g 26.97 mmol) a clear colorless solution of tetrabutylammonium fluoride 1M in THF (80.9 ml, 80.9 mmol) was added and the mixture was stirred vigorously at r.t. for 15 min (or total conversion by TLC). The reaction was quenched by addition of  $\text{H}_2\text{O}$  (4 ml) and silica gel (50 g). The crude material was concentrated and purified by flash LC (silica gel, grad hex:EtOAc 2:1 to

1:1) to yield **SAPLA3** (12.2 g, 95%) as a white solid (mixture of diastereomers).  $R_f = 0.36$  and  $0.29$  (silica, hex:CHCl<sub>3</sub>:IPA; 1:5:1).

IR (film, DCM)  $\nu$  3450-3293, 3060 and 3040, 2961, 2946, 2883, 2852, 1746, 1632, 1533, 1454, 1357, 1387, 1265, 1173, 1095, 1045, 1018 cm<sup>-1</sup>.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.71 (d,  $J = 6.8$ , 3H), 0.81 (d,  $J = 6.6$ , 3H), 0.88 (d,  $J = 6.5$ , 3H), 0.91 (d,  $J = 6.5$ , 3H), 0.94 (d,  $J = 6.5$ , 3H), 0.99 (d,  $J = 7.1$ , 3H), 1.07 (d,  $J = 6.5$ , 3H), 1.36 (d, 6.5, 3H), 1.43-1.52 (m, 2H), 1.60-1.66 (m, 1H), 1.93-2.10 (m, 3H), 2.12-2.23 (m, 2H), 3.53-3.58 (m, 1H), 3.65 (q,  $J = 7.1$ , 1H), 3.67-3.73 (m, 1H), 3.89 (q,  $J = 7.1$ , 1H), 3.96 (d,  $J = 4.2$ , 1H), 4.22 (d,  $J = 4.1$ , 1H), 4.54-4.56 (m, 1H), 4.58-4.62 (m, 1), 4.69-4.73 (m, 1H), 5.1 (d,  $J = 12.1$ , 1H), 5.18 (d,  $J = 12.1$ , 1H), 6.57 (d,  $J = 8.5$ , 1H), 6.63 (d,  $J = 8.5$ , 1H), 7.28-7.38 (m, 5H).

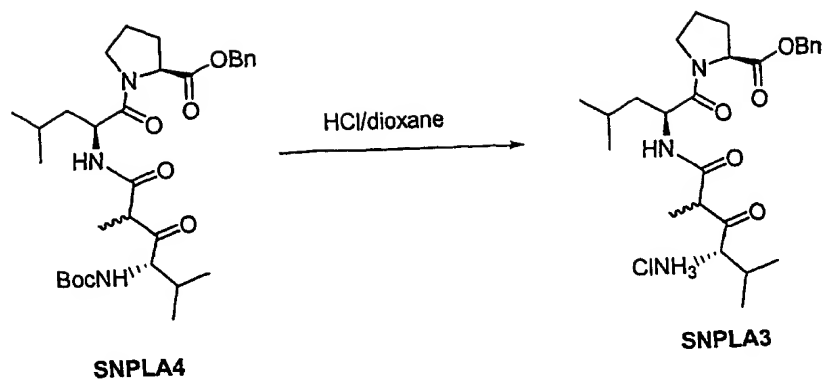
<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  14.06, 14.26, 15.85, 16.48, 20.07, 20.53, 22.02, 22.25, 25.37, 25.46, 29.45, 29.53, 31.59, 32.09, 41.13, 42.29, 49.93, 50.91, 51.02, 59.52, 67.60, 81.02, 128.78, 1.29.2, 169.48, 171.58, 172.17, 209.76.

$m/z$  (FAB) 497.4 [(M + Na)<sup>+</sup>, 12], 475.5 [(M + H)<sup>+</sup>, 100].  $m/z$  (FABHRMS) 497.263 162, C<sub>26</sub>H<sub>38</sub>N<sub>2</sub>O<sub>6</sub> requires (M+Na)<sup>+</sup> 497.262 757. Anal. Calcd for C<sub>26</sub>H<sub>38</sub>N<sub>2</sub>O<sub>6</sub>: C, 65.82; H, 8.02; N, 5.91. Found: C, 65.97; H, 8.18; N, 5.76.

#### Example 17

#### Synthesis of Aip-Leu-Pro-OBn (SNPLA3)

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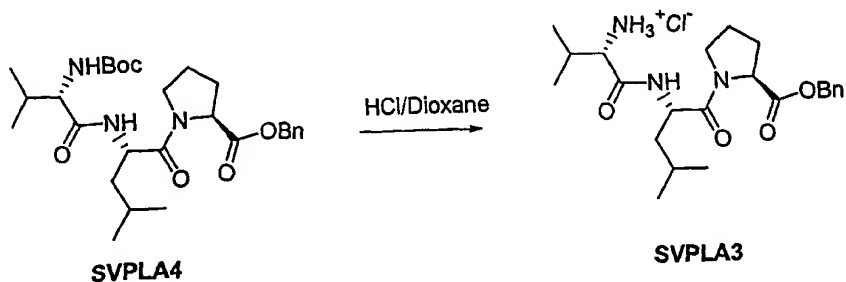


To a solution of **SNPLA4** (750 mg, 1.30 mmol) in dioxane (15 ml, anhydrous), a solution of hydrogen chloride in dioxane (39 ml, 5.3 N) was added and the mixture was stirred for 5 hours or until total conversion by TLC (disappearance of starting material). The solution was concentrated under reduced pressure and the resulting oil was chased with  $\text{CHCl}_3$  (15 ml), MTBE (15 ml) and hex (15 ml). The residue was dried under vacuum to remove residual HCl, to give a foamy solid. **SNPLA3** (660 mg, quant.) was used directly in next step with no further purification.

ESI-MS Calcd for  $\text{C}_{26}\text{H}_{39}\text{N}_3\text{O}_5$ : 473.29. Found 474.2 ( $\text{M}+\text{H}$ ) $^+$ .

### Example 18

#### Synthesis of Val-Leu-Pro-OBn (SVPLA3)





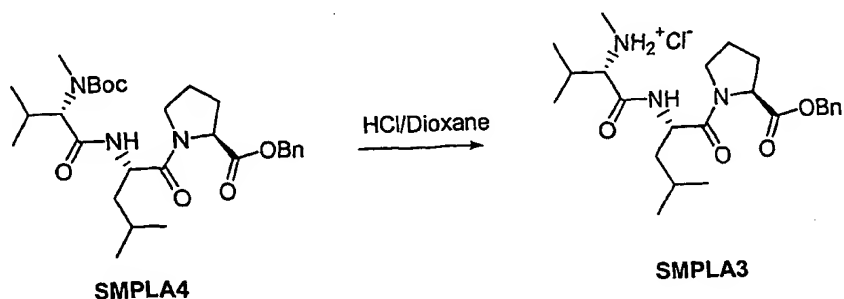
To a flask containing **SVPLA4** (215 mg, 0.41 mmol) a solution of hydrogen chloride in dioxane (1.5 ml, 5.3 N) was added and the mixture was stirred for 5 hours or until total conversion by TLC. The solution was concentrated under reduced pressure and the resulting oil was chased with  $\text{CHCl}_3$  (5 ml), MTBE (5 ml) and hex (5 ml). The residue was dried under vacuum to remove residual HCl, to give a foamy solid of **SVPLA3** (185 mg, quant.) was used directly in next step with no further purification.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.86–0.90 (m, 6H), 1.04 (d,  $J = 6.3$ , 3H), 1.12 (d,  $J = 6.3$ , 3H), 1.45–1.55 (m, 1H), 1.60–1.80 (m, 2H), 1.82–2.11 (m, 2H), 2.11–2.25 (m, 1H), 2.25–2.40 (m, 1H), 3.50–3.70 (m, 1H), 3.80–3.95 (m, 2H), 4.52–4.57 (s, 1H), 4.70–4.85 (m, 1H), 5.05 (d,  $J = 12$ , 1H), 5.20 (d,  $J = 12.3$ , 1H), 7.27–7.37 (m, 5H), 7.91 (m, 1H), 8.62 (bs, 2H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  18.57, 18.79, 21.83, 23.11, 24.50, 24.67, 24.90, 25.34, 28.92, 30.23, 33.25, 40.40, 47.04, 49.46, 49.94, 59.26, 60.02, 66.88, 128.16, 128.27, 128.51, 135.48, 167.54, 170.80, 171.94.

#### Example 19

##### Synthesis of (Me)Val-Leu-Pro-OBn (**SMPLA3**)



Following the procedure described for the synthesis of **SVPLA3**, starting from **SMPLA4** (940 mg, 1.94 mmol) the title compound (828 mg, quant.) was obtained as a white solid. This product was used directly in next step with no further purification.

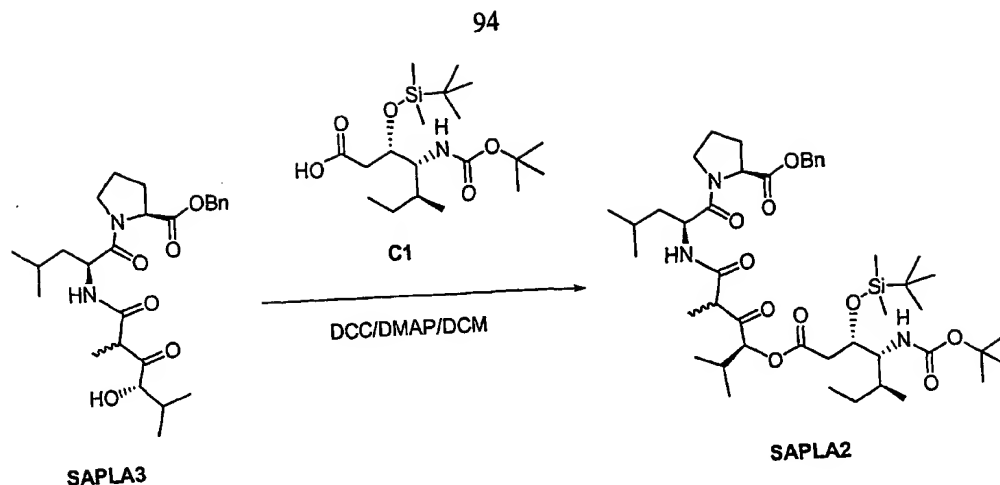
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.93 (d,  $J=6.3$ , 6H), 1.07 (d,  $J=6.3$ , 3H), 1.21 (d,  $J=6.3$ , 3H), 1.47 (m, 1H), 1.73 (m, 2H), 2.00 (m, 3H), 2.23 (m, 1H), 2.52 (m, 1H), 2.83 (bs, 3H), 3.56-3.65 (m, 2H), 3.77 (m, 1H), 4.59 (m, 1H), 4.66 (m, 1H), 5.07 (d,  $J=12.3$ , 1H), 5.19 (d,  $J=12.3$ , 1H), 7.27-7.38 (m, 5H), 7.90 (m, 1H), 9.11 (m, 0.5H), 9.61 (m, 0.5H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  13.98, 18.37, 19.57, 21.33, 22.52, 23.16, 24.74, 28.77, 29.78, 31.54, 32.54, 39.87, 46.75, 50.09, 58.91, 66.85, 122.12, 128.06, 128.24, 128.47, 135.43, 166.22, 170.73, 171.54.

ESI-MS Calcd for  $\text{C}_{24}\text{H}_{38}\text{ClN}_3\text{O}_4$ : 431.2. Found ( $m/z$ ): 432.2 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 20

#### Synthesis of Boc-Ist(TBDMS)-Hip-Leu-Pro-OBn (SAPLA2)



To a solution of **SAPLA3** (12.2 g, 25.44 mmol) in anh. DCM (75 ml) at  $-5^{\circ}\text{C}$  under Ar, DMAP (0.932 g, 7.6 mmol), **C1** (11.89 g, 30.53 mmol) and DCC (6.613 g, 32.05 mmol) were added in portions, while maintaining the temperature  $< -5^{\circ}\text{C}$  (ice-salt bath). The reaction mixture was stirred for 14 h at  $-5^{\circ}\text{C}$  and then, filtered and concentrated. The crude material was chased with ACN, cooled ( $-10^{\circ}\text{C}$ ), filtered and concentrated again. The resulting material was dissolved in EtOAc (400 ml) and washed sequentially with aq.  $\text{KHSO}_4$  (2x200 ml, 10%), brine (200 ml), aq.  $\text{NaHCO}_3$  (200 ml, sat.) and rinsed with brine (200 ml). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated at reduced pressure to afford a colourless oil which was chromatographed on silica gel eluting with a gradient of Hex-EtOAc 3:1 to 2:1, to yield **SAPLA2** (19.35 g, 90%) as a white foam (mixture of diastereomers).

IR (film, DCM)  $\nu$  3365-3200, 3069, 3038, 2959, 2930, 2882, 2857, 1746, 1688, 1640, 1533, 1456, 1389, 1258, 1171, 1086  $\text{cm}^{-1}$ .

$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.01 (s, 3H), 0.03 (s, 3H), 0.05 (s, 3H), 0.07 (s, 3H), 0.77-1.03 (m, 18H), 0.84 (s, 9H), 0.85 (s, 9H), 1.33 (d,  $J = 7.4$ , 3H), 1.32-1.36 (m, 2H), 1.49 (d,  $J = 7.5$ , 3H), 1.38-1.62 (m, 3H), 1.42

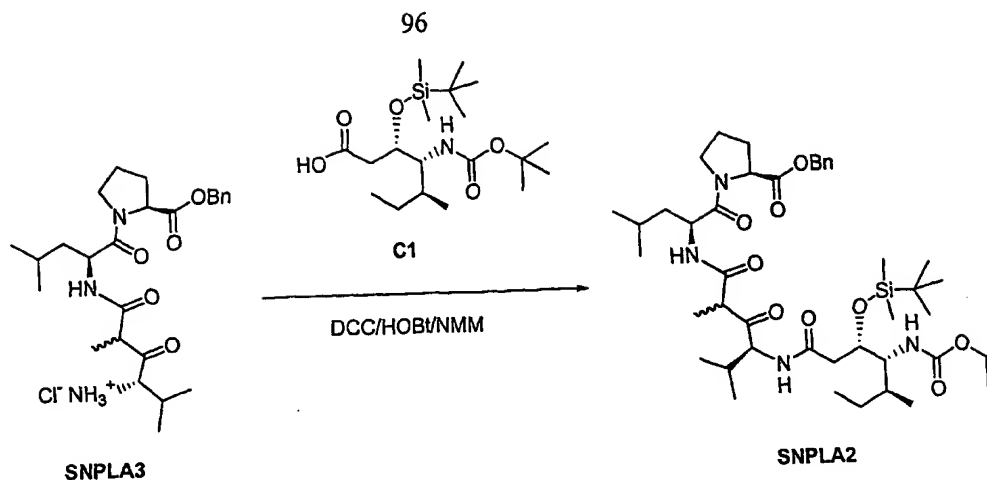
(s, 9H), 1.44 (s, 9H), 1.51-1.77 (m, 1H), 1.88-2.37 (m, 3H), 2.17-2.33 (m, 2H), 2.47-2.74 (m, 2H), 3.34-3.72 (m, 1H), 3.72-3.82 (m, 1H), 3.99-4.40 (m, 1H), 4.03-4.16 (m, 1H), 4.49 (d, J = 10.3, 1H), 4.54-4.59 (m, 1H), 4.63-4.70 (m, 2H), 4.75 (d, J = 4.5, 1H), 4.77-4.81 (m, 1H), 4.95-5.19 (m, 2H), 5.22 (d, J = 5.2, 1H), 5.32 (d, J = 10.5, 1H), 6.38 (d, J = 10.9, 1H), 6.71 (d, J = 7.4, 1H), 6.76 (d, J = 8.4, 1H), 8.60 (d, J = 9.5, 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.05, -4.49, 11.83, 12.03, 13.01, 13.51, 13.83, 14.08, 16.92, 17.10, 17.85, 19.14, 19.65, 21.57, 22.09, 22.96, 23.28, 24.36, 24.60, 24.85, 25.73, 26.97, 27.33, 28.35, 28.46, 28.93, 29.09, 29.65, 34.12, 34.16, 40.45, 40.85, 41.18, 42.20, 46.74, 46.16, 47.99, 48.34, 48.90, 49.42, 57.62, 58.81, 58.96, 60.46, 66.62, 66.88, 68.18, 69.69, 78.98, 79.24, 79.84, 82.95, 128.08-128.49, 135.48, 135.61, 155.85, 158.27, 157.44, 168.40, 169.07, 170.65, 170.86, 171.42, 171.79, 203.09, 205.97.

m/z (FAB) 846.6  $[(\text{M}+\text{H})^+, 15]$ , 746.6 (100); m/z (FABHRMS) 868.516 630,  $\text{C}_{45}\text{H}_{75}\text{N}_3\text{O}_{10}\text{Si}$  requires  $(\text{M}+\text{Na})^+$  868.511 930.

#### Example 21

#### Synthesis of Boc-Ist(TBDMS)-Aip-Leu-Pro-OBn (SNPLA2)



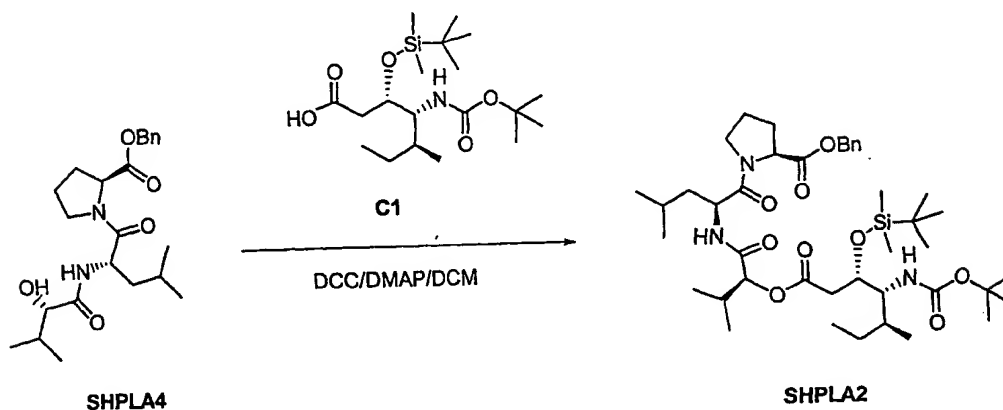
To a flask containing **SNPLA3** (chlorhydrate) (660 mg, 1.12 mmol) in DCM (15 ml, anh) at 0°C, NMM (0.19 ml) was added. After 15 min, **C1** (632 mg, 1.62 mmol), HOBT (266 mg, 1.73 mmol), and DCC (331 mg, 1.60 mmol) were added in portions. The flask was allowed to warm to room temperature and stirring was continued overnight. Crude reaction mixture was partitioned between DCM (50 ml) and aq KHSO<sub>4</sub> (2x20 ml, 10%). The organic phase was washed successively with aq. NaHCO<sub>3</sub> (2x20 ml, sat) and brine (20 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure. The resulting white solid was purified by flash LC (silica gel, gradient hex-EtOAc 4:1 to 1:1) to afford the title compound as a white solid (700 mg, 73%, mixture of diastereomers).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.05 (s, 3H), 0.08 (s, 3H), 0.09 (s, 3H), 0.68-1.05 (18H, m), 0.87 (9H, s), 1.05-1.85 (12H, m), 1.42 (9H, s), 1.85-2.10 (3H, m), 2.11-2.31 (2H, m), 2.32-2.46 (2H, m), 2.47-2.60 (m, 2H), 3.34-3.90 (2H, m), 3.93-4.30 (2H, m), 4.50-4.89 (6H, m), 4.90-5.12 (2H, m), 5.07 (d, J=12.2, 2H), 5.18 (d, J=12.2, 2H), 5.60 (1H, d, J = 9.7), 5.67 (1H, d, J = 10.2), 5.89 (1H, d, J = 11.2), 6.56 (1H, d, J = 7.3), 6.70 (1H, d, J = 8.3), 6.76 (1H, d, J = 6.8), 6.94 (d, J=6.8, 1H), 7.01-7.19 (m, 1H), 7.32 (bs, 5H), 8.17 (1H, d, J = 7.8), 8.28 (d, J=7.8, 1H).

ESI-MS Calcd for  $C_{45}H_{79}N_4O_9Si$ : 844.54. Found (m/z): 845.5 (M+H)<sup>+</sup>.

### Example 22

#### Synthesis of Boc-Ist(TBDMS)-Hiv-Leu-Pro-OBn (SHPLA2)



Following the procedure described for the synthesis of **SAPLA2**, starting from **SHPLA4** (850 mg, 2.0 mmol) and **C1** (935 mg, 2.4 mmol) the title compound was obtained (1.53 g, 97%) after purification by flash LC (silica, gradient hex-EtOAc 3:1 to 2:1).  $R_f=0.63$  (hex-EtOAc 2:1).

$^1H$  NMR (300 MHz,  $CDCl_3$ ) mixture of BocNH rotamers:  $\delta$  0.04 (s, 3H), 0.06 (s, 3H), 0.88 (s, 9H), 0.78-1.04 (m, 18H), 1.10-2.80 (m, 11H), 1.44 (s, 9H), 1.46 (s, 9H), 3.57 (m, 2H), 3.74 (m, 1H), 3.85 (m, 1H), 4.03 (m, 1H), 4.23 (d,  $J=4.8$ , 1H), 4.48 (m, 1H), 4.85 (m, 1H), 4.90 (d,  $J=10$ , 1H), 5.05 (m, 1H), 5.20 (d,  $J=10$ , 1H), 5.23 (d,  $J=10$ , 1H), 6.64 (d,  $J=6.4$ , 1H), 6.88 (d,  $J=8.6$ , 1H), 7.32 (m, 5H), 8.54 (d,  $J=8.3$ , 1H).

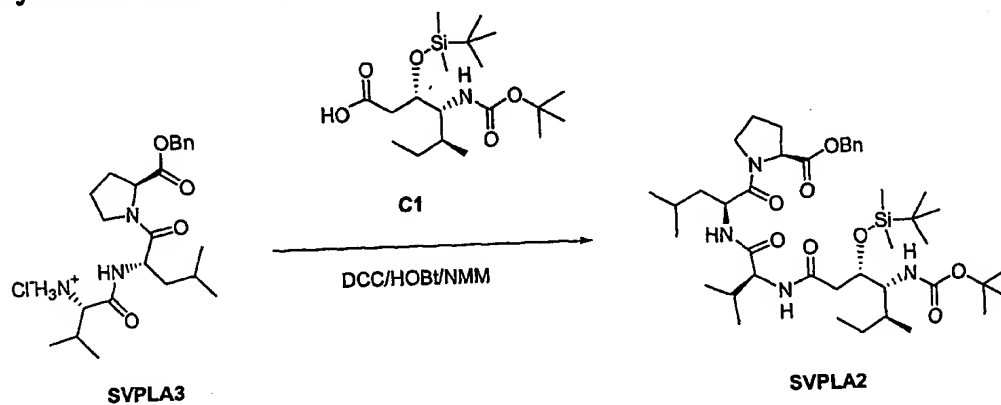
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$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  -5.14, -4.58, 11.84, 12.97, 17.78, 17.92, 18.98, 21.05, 23.11, 23.49, 25.61, 26.92, 28.36, 28.70, 30.12, 33.68, 38.72, 42.86, 46.51, 48.18, 58.67, 60.24, 66.52, 71.14, 79.40, 82.66, 127.96, 128.01, 128.33, 135.36, 157.30, 169.92, 171.10, 171.69, 171.97.

ESI-MS: Calcd for  $\text{C}_{42}\text{H}_{71}\text{N}_3\text{O}_9$ : 789.50. Found 790.5 ( $\text{M}+\text{H}$ ) $^+$ .

### Example 23

#### Synthesis of Boc-Ist(TBDMS)-Val-Leu-Pro-OBn (SVPLA2)



Following the procedure described for the synthesis of **SNPLA2**, starting from **SVPLA3** (chlorhydrate) (1.2 g, 2.64 mmol), **C1** (1.23 g, 3.17 mmol), DCC (654 mg, 3.17 mmol), HOBt (464 mg, 3.43 mmol), NMM (0.35 ml) and DCM (6 ml). The title compound was obtained as a white solid (1.87 g, 89%) after purification by flash LC (silica gel, gradient hex-EtOAc 3:1 to 2:1).

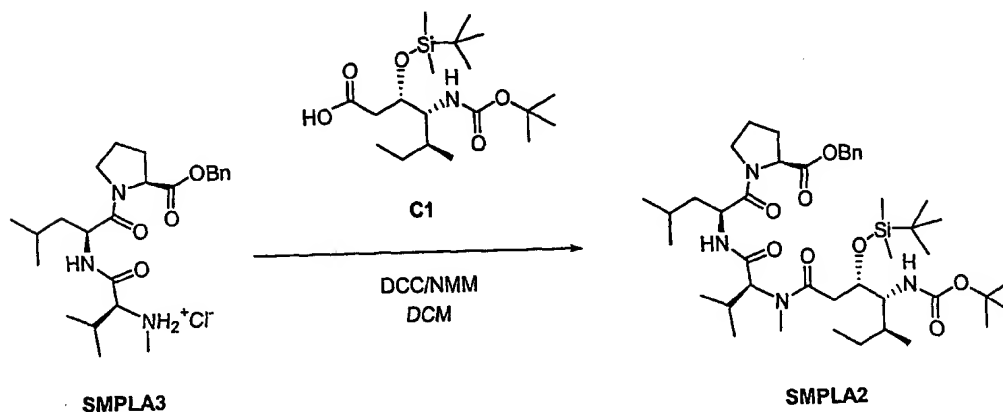
$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.07 (bs, 6 H), 0.81-0.96 (m, 27H), 1.11-1.38 (m, 3H), 1.39-1.47 (bs, 7H), 1.51 (bs, 3H), 1.58-1.70 (m, 3H), 1.70-1.84 (m, 1H), 1.86-2.60 (m, 4H), 2.28-2.58 (m, 2H), 3.58-3.62 (m,

1H), 3.62-3.73 (m, 1H), 3.73-3.90 (m, 1H), 4.05-4.12 (m, 1H), 4.13-4.19 (m, 1H), 4.19-4.23 (m, 1H), 4.49-4.54 (m, 1H), 4.77-5.06 (m, 2H), 5.18 (d,  $J = 12.3$ , 1H), 5.55 (bs, 1H), 6.44-6.61 (m, 2 H), 7.30-7.35 (m, 5 H), 7.94-7.98 (m, 1H).

ESI-MS Calcd for  $C_{42}H_{72}N_4O_8Si$ : 788.51. Found (m/z): 789.5 (M+H)<sup>+</sup>.

#### Example 24

#### Synthesis of Boc-Ist(TBDMS)-(Me)Val-Leu-Pro-OBn (SMPLA2)



To a flask containing **SMPLA3** (chlorhydrate) (176 mg, 0.38 mmol), in DCM (2 ml, anh) at 0°C, NMM (41  $\mu$ l, 0.38 mmol) was added. After 15 min, **C1** (176 mg, 0.46 mmol), and DCC (93 mg, 0.46 mmol) were added in portions. The flask was allowed to warm to room temperature and stirring was continued overnight. The reaction mixture was partitioned between DCM (10 ml) and aq  $KHSO_4$  (2x5 ml, 10%). The organic phase was washed successively with aq.  $NaHCO_3$  (2x5 ml, sat), brine (5 ml), dried ( $Na_2SO_4$ ), filtered and concentrated under reduced pressure. The



resulting white solid was purified by flash LC (silica, gradient Hex-EtOAc 3:1 to 2:1) to give **SMPLA2** (127 mg, 42%) as a white solid.  $R_f=0.51$  (Hex-EtOAc 1:1).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  -0.08 (s, 3 H), 0.05 (s, 3H), 0.75-0.86 (m, 27H), 1.00-1.43 (m, 11H), 1.52-1.65 (m, 1H), 1.68-1.80 (m, 1H), 1.83-2.01 (m, 3H), 2.08-2.24 (m, 2H), 2.40 (m, 2 H), 2.87 (s, 3H), 3.50-3.57 (m, 3H), 3.71-3.76 (m, 1H), 4.29 (m, 1H), 4.47-4.63 (m, 4H), 5.01 (d,  $J=12.9$ , 1H), 5.11 (d,  $J=12.9$ , 1H), 6.41 (d,  $J=7.8$ , 0.5 H), 6.62 (d,  $J=7.8$ , 1 H), 7.01 (d,  $J=7.8$ , 0.5 H), 7.23-7.28 (m, 5 H).

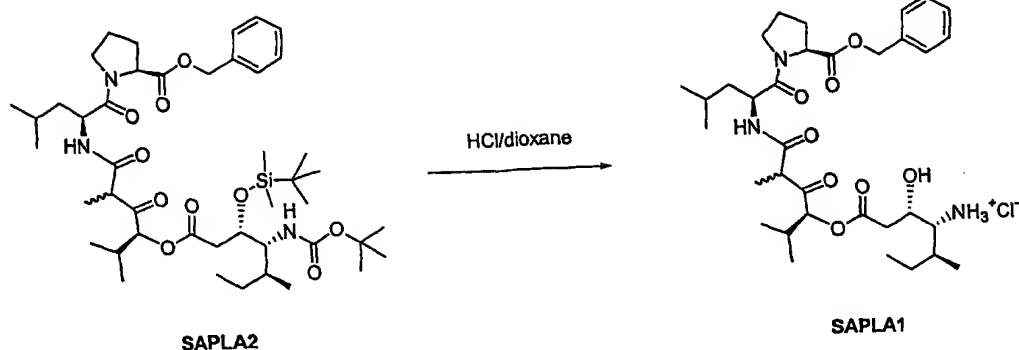
$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.56, 13.57, 13.98, 17.83, 18.87, 19.46, 21.77, 22.94, 23.11, 24.50, 24.71, 24.80, 25.64, 25.76, 25.97, 27.29, 28.18, 28.75, 30.40, 34.18, 39.28, 40.85, 46.58, 48.61, 57.03, 58.65, 62.26, 66.63, 69.21, 78.72, 127.92, 128.05, 128.11, 128.33, 135.42, 155.91, 169.76, 170.48, 171.56, 172.01.

ESI-MS Calcd for  $\text{C}_{43}\text{H}_{74}\text{N}_4\text{O}_8\text{Si}$ : 802.53. Found (m/z): 825.5 (M+Na) $^+$ .

#### Example 25

#### Synthesis of Ist-Hip-Leu-Pro-OBn (SAPLA1)

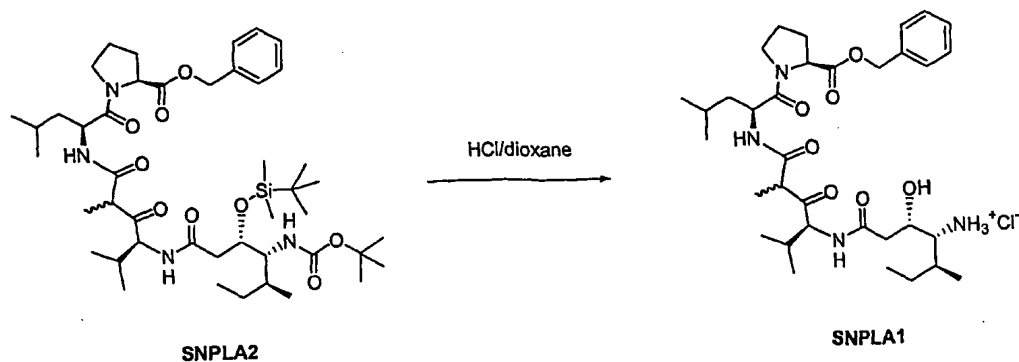
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To a solution containing **SAPLA2** (19.32 g, 22.8 mmol) in anh. dioxane (78 ml), a solution of hydrochloric acid in anhydrous dioxane (4.2 N, 220 ml, 924 mmol) was added. The resulting solution was stirred at 21°C for 4.30 h or until complete disappearance of the starting material (TLC). Then, the solution was concentrated under reduced pressure. The residue was dissolved in DCM (25 ml) and concentrated to remove residual HCl. The resulting residue was dried under vacuum until complete elimination of free HCl (3 h) to give 17.3 g of **SAPLA1** (15.1 g, quant) as a white foam (mixture of diastereomers).

#### Example 26

##### Synthesis of Ist-Aip-Leu-Pro-OBn (SNPLA1)



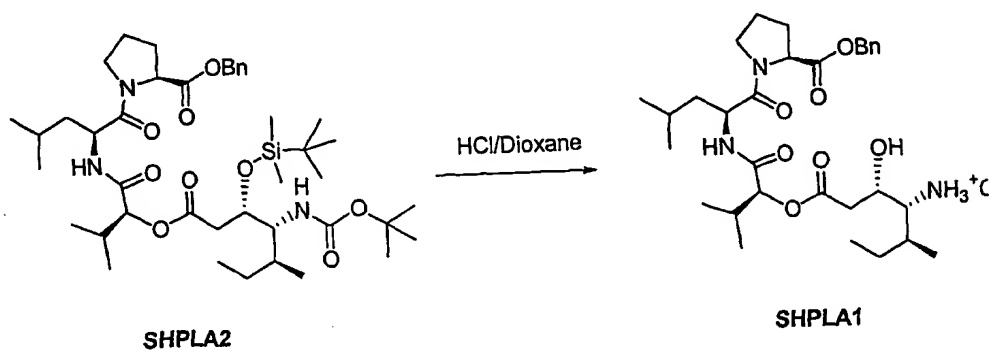
Following the procedure described for synthesis of **SAPLA1**, starting from **SNPLA2** (700 mg, 0.82 mmol), the title compound was obtained as a white solid (545 mg, quant.) after precipitation with Et<sub>2</sub>O (mixture of diastereomers).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.86-1.04 (m, 18H), 1.02-1.22 (m, 3H), 1.23-1.58 (m, 5H), 1.60-1.80 (m, 2H), 1.82-2.01 (m, 3H), 2.24 (m, 2H), 2.40-2.85 (m, 2H), 3.24 (m, 1H), 3.45 (m, 1H), 3.60 (m, 1H), 3.70-4.05 (m, 2H), 4.46 (m, 2H), 4.47-4.75 (m, 2H), 5.10 (bs, 2H), 7.34 (bs, 5H), 7.98 (bs, 1H), 8.10 (bs, 1H).

ESI-MS Calcd for C<sub>34</sub>H<sub>54</sub>N<sub>4</sub>O<sub>7</sub>: 630.40. Found (m/z): 631.4 (M+H)<sup>+</sup>.

#### Example 27

##### Synthesis of Ist-Hiv-Leu-Pro-OBn (**SHPLA1**)



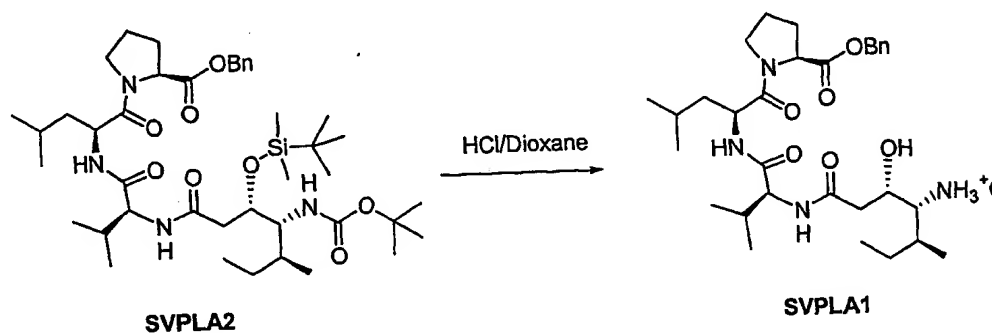
Following the procedure described for synthesis of **SAPLA1**, starting from **SHPLA2** (1.53 g, 1.94 mmol), the title compound (1.12 g, quant.) was obtained as a white solid after precipitation with Et<sub>2</sub>O.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.86-1.04 (m, 18H), 1.10-1.22 (m, 3H), 1.42 (m, 2H), 1.70 (m, 2H), 1.97 (m, 3H), 2.24 (m, 2H), 2.83 (m, 1H), 2.97 (m, 1H), 3.34 (m, 1H), 3.61 (m, 1H), 3.75 (m, 1H), 3.90 (m, 1H), 4.56 (m, 2H), 4.75 (m, 1H), 5.04 (d,  $J=11$ , 1H), 5.18 (d,  $J=11$ , 1H), 7.34 (bs, 5H), 8.21 (bs, 3H).

ESI-MS Calcd for  $\text{C}_{31}\text{H}_{49}\text{N}_3\text{O}_7$ : 575.36. Found ( $m/z$ ): 576.3 ( $\text{M}+\text{H}$ ) $^+$ .

### Example 28

#### Synthesis of Ist-Val-Leu-Pro-OBn (SVPLA1)



Following the procedure described for synthesis of **SAPLA1**, starting from **SVPLA2** (1.87 g, 2.36 mmol), the title compound (1.40 g, quant.) was obtained as a white solid after precipitation with  $\text{Et}_2\text{O}$ .

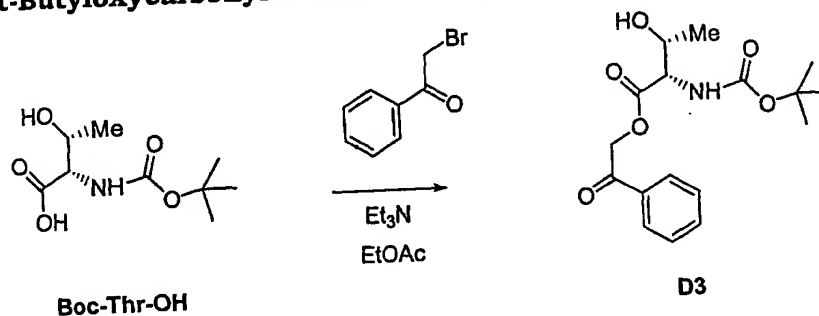
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-1.01 (m, 15H), 1.01-1.10 (m, 3H), 1.20-1.79 (m, 5H), 1.81-2.05 (m, 4H), 2.05-2.15 (m, 2H), 2.50-2.68 (m, 2H), 2.82-3.1 (m, 1H), 3.20-3.35 (m, 1H), 3.50-3.70 (m, 1H), 3.80-3.90 (m, 1H), 4.18-4.30 (m, 1H), 4.35-4.45 (m, 1H), 4.45-4.55 (m, 1H), 4.60-4.70 (m, 1H), 5.02 (d,  $J = 12.3$ , 1H), 5.15 (d,  $J = 12.3$ , 1H), 7.28-7.38 (m, 5H), 7.5 (bs, 1H), 7.9 (bs, 3H), 8.15 (bs, 1H).

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$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  10.81, 14.67, 18.29, 19.32, 21.48, 23.14, 24.48, 24.76, 25.81, 30.29, 33.41, 40.31, 46.96, 49.35, 59.14, 59.94, 60.67, 66.94, 128.11, 128.32, 128.54, 135.32, 171.58, 171.74, 171.80, 172.57.

ESI-MS Calcd for  $\text{C}_{31}\text{H}_{50}\text{N}_4\text{O}_6$ : 574.35. Found ( $m/z$ ): 575.3 ( $\text{M}+\text{H}$ ) $^+$ .

## Example 29

**N-tert-Butyloxycarbonylthreonine Phenacyl ester (D3)**

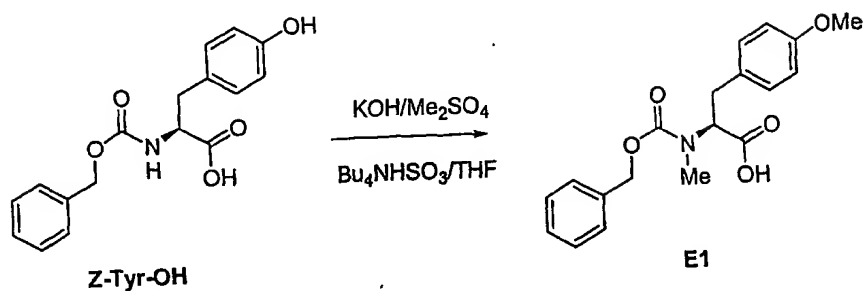
To a stirred suspension of **Boc-Thr-OH** (21.91 g, 0.1 mol) in  $\text{EtOAc}$  (200 ml) at  $0^\circ\text{C}$ , TEA (14 ml, 0.1 mol) and bromoacetophenone (19.0 g, 0.1 mol) were added. The reaction mixture was allowed to warm to  $20^\circ\text{C}$ , stirred for 2 days and then diluted with  $\text{EtOAc}$  (500 ml). After washing successively with aq HCl (200 ml, 0.1 N),  $\text{H}_2\text{O}$  (100 ml), aq  $\text{NaHCO}_3$  (200 ml, 1 N) and brine (200 ml), drying ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated in vacuo, the residue was triturated with  $\text{Et}_2\text{O}$  and filtered. The resulting solid was dried in the dark to yield **D3** (28.6 g, 85%).  $R_f$  = 0.55 (hex- $\text{EtOAc}$  1:1, silica); M.p. =  $114.2^\circ\text{C}$ ;  $[\alpha]_D^{22}$   $-29.4$  (c 2,  $\text{EtOAc}$ ).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  = 1.31 (d,  $J$  = 6.6, 3H), 1.46 (s, 9H), 3.77 (br d, OH), 4.44 (dd,  $J$  = 9.6, 1H), 4.6 (q, 1H), 5.34 (d,  $J$  = 16.5, 1H)

5.37 (br d, OH) 5.68 (d,  $J = 16.8$ , 1H), 7.51 (t, 2H), 7.65 (t, 1H), 7.92 (dd, 2H).

### Example 30

#### **N-Benzoyloxycarbonyl-N,O-Dimethyl-L-tyrosine (E1)**

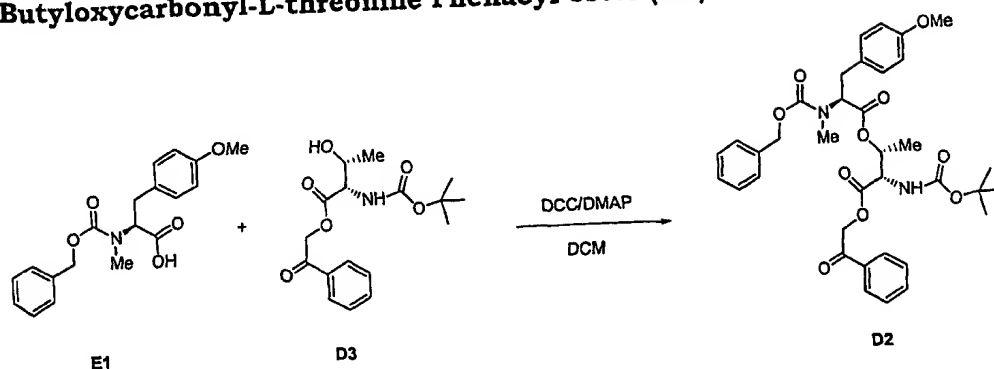


To a stirred solution of Z-Tyr-OH (63.24 g, 200 mmol) in THF (900 ml) at 0° C was added finely powdered KOH (112.72 g, 2 mol) in portions, followed by the addition of tetrabutylammonium hydrogen sulfate (6.36 g, 10% by weight). Then, dimethyl sulfate (127.2 ml, 1.33 mol) was added dropwise over 30 min, while maintaining the reaction mixture below 4° C. The reaction was stirred for an additional 30 min and H<sub>2</sub>O (950 ml) was added. After stirring 5 h at 0° C, the reaction was diluted with ether (1500 ml), the aqueous layer was separated, and the organic layer was extracted with aq NaHCO<sub>3</sub> (2x 500 ml, sat). The combined aqueous layers were acidified to pH 1 with aq 1M KHSO<sub>4</sub>, and extracted with EtOAc (5x500 ml). The organic layers were combined, dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The residue was precipitated with ethyl ether and filtered to give **E1** as a white solid (53.85 g, 78%).

$[\alpha]_D^{22} -57.16$  (c 2.23 CHCl<sub>3</sub>) [lit  $[\alpha]_D -48$  (c= 2,23 CHCl<sub>3</sub>). JACS, 112,21, 1990).

## Example 31

**O-(Benzyloxycarbonyl-N,O-dimethyl-L-tyrosyl)-N-tert-Butyloxycarbonyl-L-threonine Phenacyl ester (D2)**

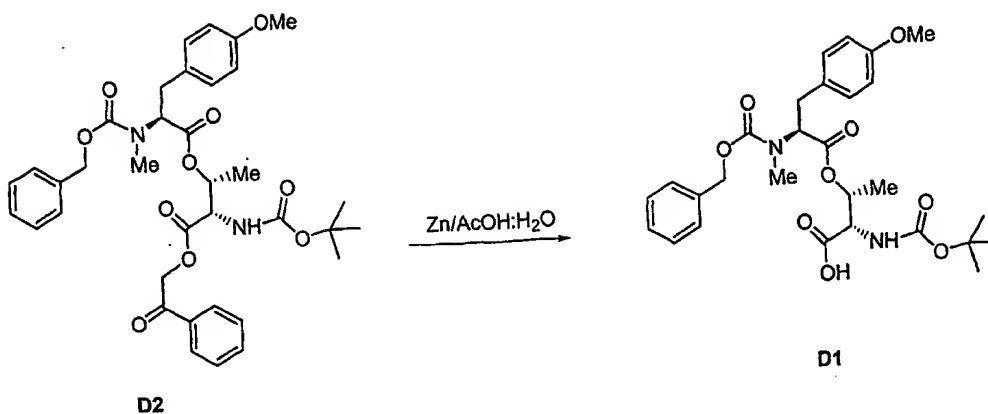


To a solution of **D3** (33.72 g, 100 mmol) in DCM at 0° C, DMAP (3.66 g, 30 mmol), and **E1** (34.33 g, 100 mmol) were added. After stirring 10 min at 0° C, DCC (22.7 g, 110 mmol) was added. The reaction mixture was allowed to warm to room temperature and stirred overnight. Then the mixture was filtered and the filtrate concentrated to dryness. The residue was chased with ACN (100 ml), filtered again and the filtrate was concentrated. The residue was dissolved in EtOAc (200 ml) and partitioned successively between aq KHSO<sub>4</sub> (100 ml, 10%), aq NaHCO<sub>3</sub> (100 ml, sat.) and brine (100 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The residue was purified by flash LC (silica gel, grad EtOAc-Hex 1:4 to 1:2) to yield **D2** (65.5 g, 98%).  $[\alpha]_D^{22}$  -39.56 (c 1.06 CHCl<sub>3</sub>); R<sub>f</sub> = 0.55 (EtOAc:Hex 1:1).

## Example 32

**O-(Benzyloxycarbonyl-N,O-dimethyl-L-tyrosyl)-N-tert-Butyloxycarbonyl-L-threonine (D1)**

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To a homogeneous solution of **D2** (24.49 g, 38.4 mmol) in aq AcOH (211 ml, 90%) at 0° C, powdered Zn was added (18.65g, 288.3 mmol). The resulting mixture was stirred at 0°C for 3h until disappearance of the starting material (followed by TLC). The reaction mixture was filtered over celite and washing with EtOAc (200 ml). The filtrate was concentrated at reduced pressure and the residue was chased with Et<sub>2</sub>O (200 ml) and filtered. The filtrate was successively partitioned between aq KHSO<sub>4</sub> (100ml, 10%) and brine (100 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated to give an oil which was purified by flash LC (Lichroprep RPC18, ACN:H<sub>2</sub>O 1:1 (800 ml, then 7:3 (600 ml)) to yield **D1** (15.53g, 74%) as a white solid.  $[\alpha]_{\text{D}}^{24}$  -27.6 (c 2.187, DCM); lit  $[\alpha]_{\text{D}}$  -20.5 (c 2, DCM). JOC, 62, 2, 1997. R<sub>f</sub> = 0.58 [ACN/H<sub>2</sub>O (7:3)].

IR (film, DCM)  $\nu$  3400, 3050, 2900, 1715, 1613, 1514, 1456, 1402, 1368, 1248, 1165, 1061, 1036 cm<sup>-1</sup>.

<sup>1</sup>H NMR (200 MHz, CDCl<sub>3</sub>)  $\delta$  1.29 (d, J = 6.5, 3H), 1.45 (s, 9H), 2.74 (s, 3H), 2.75 (s, 3H), 2.76-3.31 (m, 2H), 3.77 (s, 3H), 4.42-4.52 (m, 1H), 4.66-4.83 (m, 1H), 5.01-5.16 (m, 2H), 5.30-5.53 (m, 2H), 6.72-6.81 (m, 2H), 6.95-7.09 (m, 2H), 7.35 (bs, 5H).

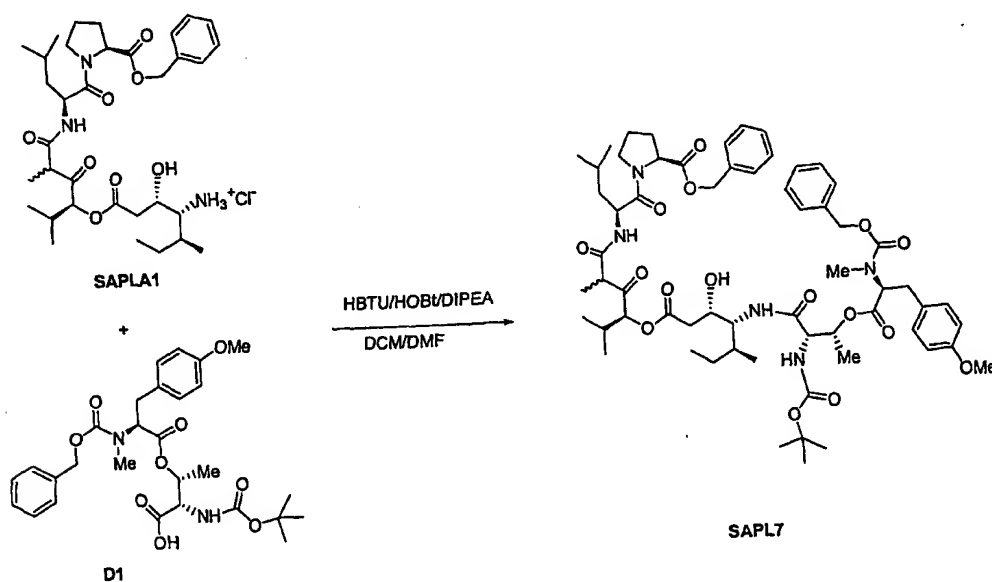


$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  16.44, 16.82, 28.23, 31.71, 31.97, 33.82, 33.68, 55.14, 56.87, 56.75, 60.39, 60.58, 67.51, 67.76, 71.83, 72.47, 80.40, 113.91, 127.59, 128.69, 129.77, 136.42, 156.00, 156.19, 156.71, 158.31, 159.47, 169.78.

$m/z$  (FAB) 567.1  $[(M+\text{Na})^+, 46]$ , 545.1  $[(M+\text{H})^+, 7]$ , 445.1 (100);  $m/z$  (FABHRMS) 567.233 280,  $\text{C}_{28}\text{H}_{35}\text{N}_2\text{O}_9$  requires  $(M+\text{Na})^+$  567.231 851.

### Example 33

#### Synthesis of Boc-Thr(Z-N(Me)-O(Me)-Tyr)-Ist-Hip-Leu-Pro-OBn (SAPL7).



To flask containing HBTU (9.079 g, 23.9 mmol), HOBt (3.490 g, 22.8 mmol), **SAPLA1** (15.258 g, 22.8 mmol) and **D1** (12.417 g, 22.8 mmol), a solution of anh DCM (296 mL) and anh DMF (148 mL) were

cannulated under Ar at -5 °C. After 5 min of stirring, DIPEA (15.9 mL, 91.2 mmol) was added dropwise by syringe, while maintaining the temperature < -5 °C. The resulting reaction mixture was stirred for 21 h at -5 °C. MTBE (300 mL) and KHSO<sub>4</sub> (200 mL, 10%) were added, and the resulting mixture was filtered off and concentrated up to 300 mL. Additional MTBE (200 mL) was added, the layers were separated, and the organic phase was treated sequentially with aq. KHSO<sub>4</sub> (200 mL, 10%), brine (200 mL), aq. NaHSO<sub>4</sub> (200 mL, sat.) and rinse with brine (200 mL). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure to afford a yellow oil (30 g). The oil was dissolved in MTBE and treated with hex while stirring. Solid precipitated and more hex was added. The solid was filtered to yield **SAPL7** (18.33 g, 69% yield) as a white solid. This product is a mixture of two diastereomers. R<sub>f</sub> = 0.80 and 0.59 (hex:EtOAc 1:2).

IR (film, DCM)  $\nu$  3350, 2961, 2927, 2893, 1744, 1688, 1638, 1514, 1454, 1368, 1304, 1248, 1171, 1067, 1036 cm<sup>-1</sup>.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.74-0.92 (m, 18H), 1.05-1.15 (m, 2H), 1.18-1.20 (m, 2H), 1.23 (d, J = 6.8, 3H), 1.25 (d, J = 6.8, 3H), 1.29 (d, J = 6.9, 3H), 1.42 (s, 9H), 1.45 (s, 9H), 1.50-1.66 (m, 3H), 1.89-2.02 (m, 4H), 2.17-2.25 (m, 2H), 2.37-2.42 (m, 1H), 2.81 (s, 3H), 2.88 (s, 3H), 2.91 (s, 3H), 2.95 (s, 3H), 2.84-2.93 (m, 2H), 3.17-3.25 (m, 1H), 3.53-3.59 (m, 1H), 3.75 (s, 3H), 3.88-3.98 (m, 4H), 4.49 (d, J = 3.1, 1H), 4.51 (d, J = 3.1, 1H), 4.53-4.57 (m, 1H), 4.68-4.72 (m, 1H), 4.96-4.99 (m, 1H), 5.02-5.33 (m, 4H), 5.02 (d, J = 3.2, 1H), 5.23 (d, J = 3.1, 1H), 5.26-5.33 (m, 1H), 5.47 (1d, J = 9.5, 1H), 6.74 (d, J = 7.8, 2H), 6.77 (d, J = 7.7, 2H), 7.08 (d, J = 7.7, 2H), 7.17 (d, J = 7.5, 1H), 7.21 (d, J = 9.5, 1H), 7.23-7.36 (m, 10H), 7.75 (d, J = 7.9, 1H), 7.79 (d, J = 8.2, 1H).

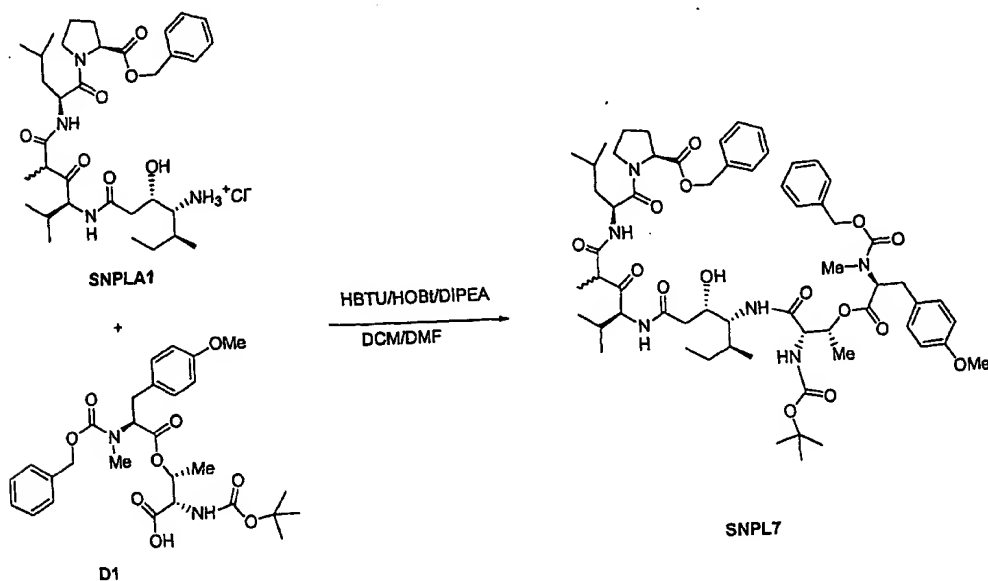
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$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.95, 13.27, 15.16, 16.47, 17.33, 18.79, 21.28, 23.65, 24.65, 24.72, 27.09, 28.08, 28.93, 31.20, 31.32, 33.62, 33.98, 38.38, 41.01, 47.12, 49.38, 54.96, 55.17, 57.89, 58.83, 60.01, 60.16, 67.18, 71.05, 71.32, 80.34, 81.24, 113.89, 127.51, 128.59, 129.69, 129.77, 135.52, 136.77, 156.93, 158.27, 169.87, 170.62, 171.15, 171.85, 172.39, 204.88.

$m/z$ (FAB) 1181.2  $[(\text{M}+\text{Na})^+, 20]$ , 1159.2  $[(\text{M}+\text{H})^+, 80]$ , 1059.2 (100).  
 Anal. Calcd for  $\text{C}_{62}\text{H}_{87}\text{N}_5\text{O}_{16}$ : C, 64.30; H, 7.52; N, 6.05. Found: C, 64.14; H, 7.66; N, 5.95

## Example 34

**Synthesis of Boc-Thr(Z-N(Me)-O(Me)-Tyr)-Ist-Aip-Leu-Pro-OBn (SNPL7).**



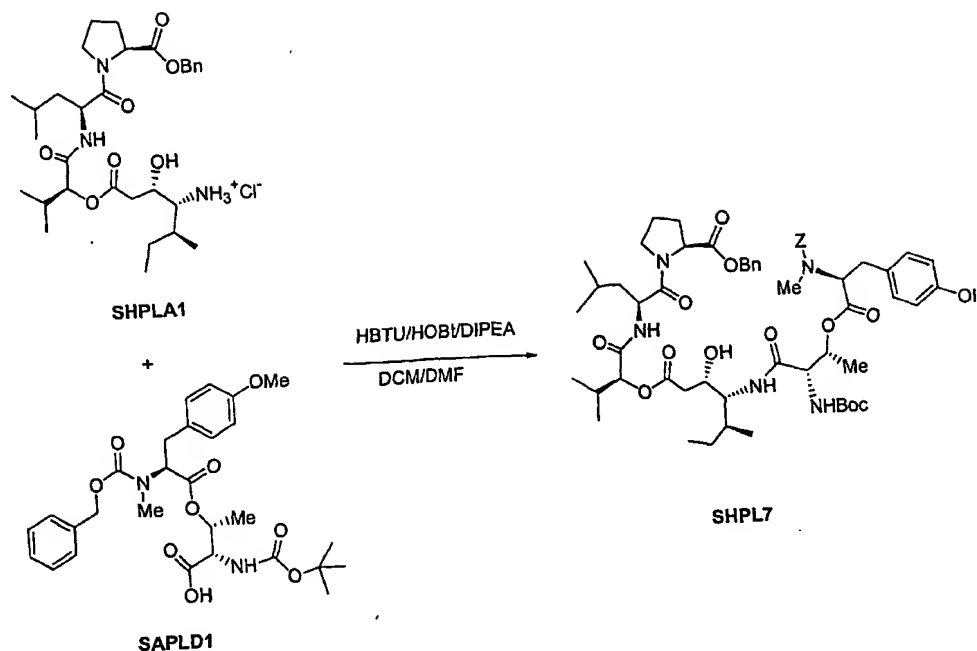
Following the procedure described for synthesis of **SAPL7**, starting from **SNPLA1** (150 mg, 0.22 mmol) and **D1** (122 mg, 0.22 mmol), the title compound was obtained as a white solid (130 mg, 51%) after purification by flash LC (silica gel, gradient hex-EtOAc 2:1 to 1:3) (mixture of diastereomers).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.74-1.03 (m, 18H), 1.16-1.37 (m, 10H), 1.45 (s, 9H), 1.68 (m, 3H), 1.99 (m, 4H), 2.22 (m, 2H), 2.48 (m, 1H), 2.82 (s, 3H), 2.84-3.10 (m, 2H), 3.19 (m, 1H), 3.51-3.69 (m, 2H), 3.75 (s, 3H), 3.72-4.02 (m, 3H), 4.18 (m, 1H), 4.50-4.73 (m, 4H), 5.00-5.27 (m, 5H), 5.49 (m, 2H), 6.54 (d,  $J=9.2$ , 1H), 6.78 (d,  $J = 6.8$ , 2H), 7.02 (d,  $J = 6.8$ , 2H), 7.18 (m, 1H), 7.23-7.36 (m, 10H), 7.52 (d,  $J = 6.8$ , 1H).

ESI-MS Calcd for  $\text{C}_{62}\text{H}_{88}\text{N}_6\text{O}_{15}$ : 1156.63. Found 1158.3 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 35

Synthesis of **Boc-Thr(Z-N(Me)-O(Me)-Tyr)-Ist-Hiv-Leu-Pro-OBn (SHPL7)**.



Following the procedure described for synthesis of **SAPL7**, starting from **SHPLA1** (1.12 g, 1.94 mmol) and **SAPLD1** (544.6 mg, 1.94 mmol), the title compound (1.045 g, 61%) was obtained as a white solid after purification by flash LC (silica gel, gradient hex-EtOAc 1:1 to 1:2).  $R_f$  = 0.46 (hex-EtOAc 1:2).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.74-1.02 (m, 18H), 1.20 (m, 5H), 1.40 (m, 3H), 1.46 (s, 9H), 1.62 (m, 2H), 1.82-2.20 (m, 3H), 2.20 (m, 2H), 2.50 (m, 1H), 2.78 (s, 3H), 2.90 (m, 1H), 3.20 (m, 1H), 3.58 (m, 1H), 3.67 (s, 1H), 3.79 (m, 1H), 3.88 (m, 1H), 4.06 (m, 2H), 4.40 (m, 2H), 4.82 (m, 2H), 4.94 (m, 1H), 4.98 (m, 1H), 5.08 (m, 1H), 5.28 (m, 3H), 5.56 (d,  $J=6.2$ , 1H), 6.84 (d,  $J=8.3$ , 2H), 6.98 (d,  $J=6.5$ , 1H), 7.07 (d,  $J=8.3$ , 2H), 7.34 (m, 10H), 7.52 (d,  $J=6.2$ , 1H).

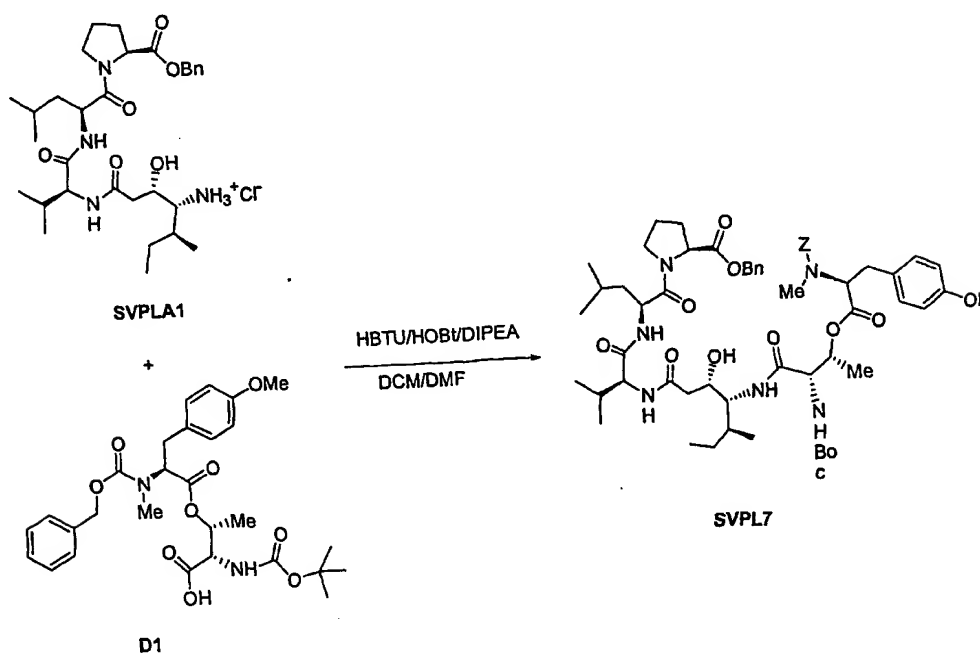
113

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.27, 13.77, 13.95, 16.75, 17.46, 18.31, 20.78, 20.96, 23.09, 24.35, 24.53, 26.74, 27.93, 28.63, 30.17, 33.76, 39.06, 40.01, 46.69, 48.17, 54.89, 57.04, 57.68, 58.65, 60.20, 60.60, 66.74, 67.08, 68.31, 70.30, 78.49, 79.90, 113.62, 127.36, 127.70, 128.14, 128.18, 128.31, 129.64, 135.19, 136.31, 155.86, 156.61, 158.09, 169.77, 170.70, 171.06, 171.17, 171.78.

ESI-MS Calcd for  $\text{C}_{59}\text{H}_{83}\text{N}_5\text{O}_{15}$ : 1101.59. Found 1102.7 ( $\text{M}+\text{H}$ ) $^+$ .

### Example 36

#### Synthesis of Boc-Thr(Z-N(Me)-O(Me)-Tyr)-Ist-Val-Leu-Pro-Bn (SVPL7).



Following the procedure described for synthesis of **SAPL7**, starting from **SVPLA1** (1.44 g, 2.37 mmol) and **D1** (1.29 g, 2.37 mmol), the title

compound (1.96 g, 75%) was obtained as a white solid after purification by flash LC (silica gel, gradient hex-EtOAc 2:1 to 1:3).  $R_f = 0.56$  (EtOAc).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.83-0.95 (m, 15H), 1.0-1.22 (m, 4H), 1.23-1.44 (m, 9H), 1.60-1.65 (m, 1H), 1.87-2.01 (m, 4H), 2.09-2.20 (m, 3H), 2.77 (bs, 8H), 2.84-3.01 (m, 1H), 3.17-3.24 (m, 1H), 3.51-3.60 (m, 1H), 3.73 (s, 3H), 3.80-3.90 (m, 2H), 4.03-4.15 (m, 1H), 4.25-4.40 (m, 2H), 4.40-4.52 (m, 1H), 4.70-4.80 (m, 2H), 5.00-5.26 (m, 4H), 5.34-5.36 (m, 1H), 5.58 (m, 1H), 6.75 (d, 2H,  $J = 7.8$ ), 6.96-7.09 (m, 1H), 7.04 (d, 2H,  $J = 8.1$ ), 7.04-7.12 (m, 1H), 7.16-7.20 (m, 1H), 7.18-7.30 (m, 10H).

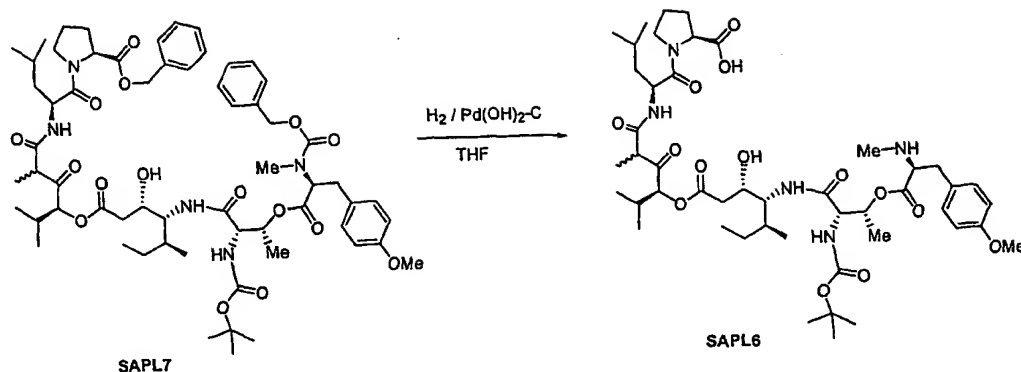
$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.43, 13.63, 17.17, 18.35, 19.21, 21.40, 23.23, 24.49, 24.67, 26.87, 28.10, 28.79, 30.48, 32.11, 33.84, 38.47, 40.37, 41.22, 46.80, 48.61, 55.04, 56.88, 57.95, 58.75, 59.25, 60.82, 66.87, 67.33, 69.40, 70.50, 76.44, 80.45, 113.58, 127.51, 127.74, 127.88, 128.10, 128.25, 128.33, 128.46, 128.72, 129.64, 129.77, 135.35, 136.37, 156.77, 158.29, 169.83, 170.57, 171.3, 171.4, 172.81.

ESI-MS Calcd for  $\text{C}_{59}\text{H}_{84}\text{N}_6\text{O}_{14}$ : 1100.60. Found (m/z): 1101.7 (M+H) $^+$ .

#### Example 37

**Synthesis of Boc-Thr(N(Me)-O(Me)-Tyr)-Ist-Hip-Leu-Pro-OH (SAPL6).**

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To a solution of **SAPL7** (18.33 g, 15.8 mmol) in THF (free of stabilizer, 500 mL) degassed and purged with argon, Pd(OH)<sub>2</sub>-C (20% Pd, 7.33 g, 40% w/w). The mixture was stirred under H<sub>2</sub> (1 atm) for 20 h, then filtered over a 0.45 μ teflon filter and concentrated under reduced pressure to give a white solid. Toluene (30 mL) was added, and concentrated again under reduced pressure and high vacuo to give **SAPL6** (14.78 g, quant) as a white solid.

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.79-1.08 (m, 18H), 1.09-1.18 (m, 3H), 1.26 (bs, 3H), 1.29 (d, J = 7.1, 3H), 1.47 (s, 9H), 1.50-1.66 (m, 3H), 1.84-1.94 (m, 1H), 1.90-2.28 (m, 4H), 2.35-2.50 (m, 4H), 2.30-2.35 (m, 1H), 2.44-3.18 (m, 4H), 2.60 (m, 3H), 3.53-3.61 (m, 1H), 3.77 (s, 3H), 3.88-4.07 (m, 4H), 4.12-4.72 (m, 4H), 5.18-5.24 (m, 1H), 5.24 (bs, 1H), 6.84 (d, J = 7.9, 2H), 7.08 (d, J = 8.0, 2H), 7.13 (d, J = 8.2, 1H), 7.18 (d, J = 8.2, 1H), 7.62-7.68 (bs, 1H).

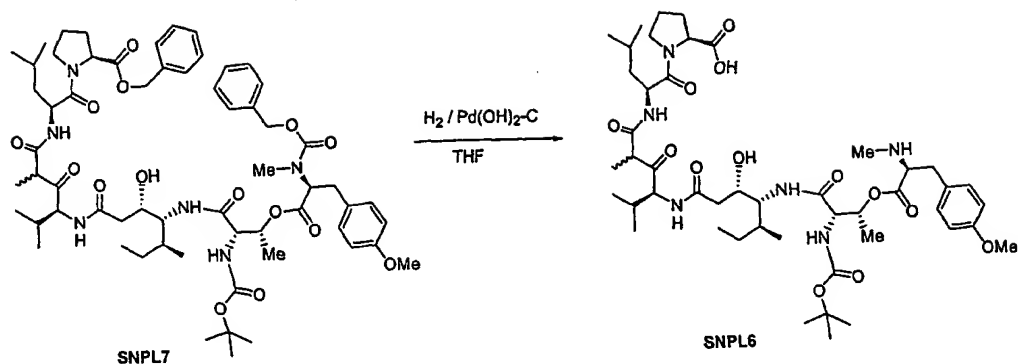
m/z (FAB) 972.7 [(M+K)<sup>+</sup>, 33], 934.9 (M)<sup>+</sup>, 100].

### Example 38

#### Synthesis of Boc-Thr(N(Me)-O(Me)-Tyr)-Ist-Aip-Leu-Pro-OH (SNPL6).



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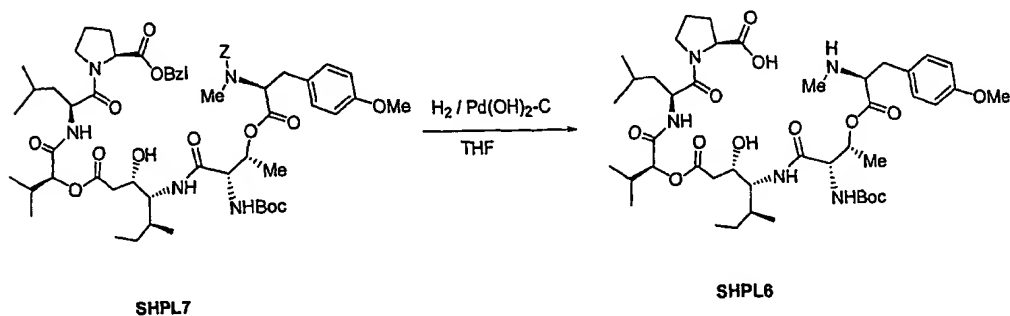


To a solution of **SNPL7** (130 mg, 0.11 mmol) in a mixture IPA:H<sub>2</sub>O (2:1, 4ml :2 ml) degassed and purged with argon, Pd(OH)<sub>2</sub>-C (20% Pd, 45 mg, 35% w/w). The mixture was stirred under H<sub>2</sub> (1 atm) for 20 h, then filtered over a 0.45  $\mu$  teflon filter and concentrated under reduced pressure to give a white solid. IPA (10 ml) was added, and concentrated again under reduced pressure and high vacuo to give **SNPL6** (100 mg, quant) as a white solid.

ESI-MS Calcd for C<sub>47</sub>H<sub>76</sub>N<sub>6</sub>O<sub>13</sub>: 932.55. Found 934.0 (M+H)<sup>+</sup>.

### Example 39

#### Synthesis of Boc-Thr(N(Me)-O(Me)-Tyr)-Ist-Hiv-Leu-Pro-OH (**SHPL6**).

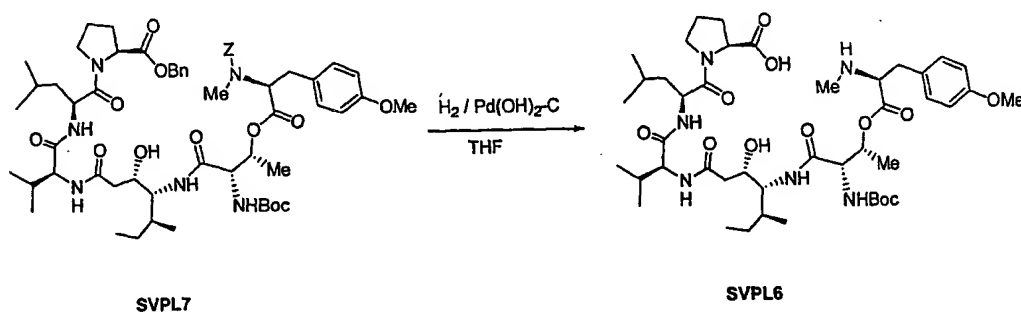


Following the procedure described for synthesis of **SAPL6**, starting from **SHPL7** (1.045 g, 0.95 mmol). The title compound (825 g, 99%) was obtained as a white solid.

ESI-MS Calcd for  $C_{44}H_{71}N_5O_{13}$ : 877.50. Found 878.5 (M+H)<sup>+</sup>.

#### Example 40

##### Synthesis of Boc-Thr(N(Me)-O(Me)-Tyr)-Ist-Val-Leu-Pro-OH (SVPL6).



Following the procedure described for synthesis of **SAPL6**, starting from **SVPL7** (250 mg, 0.23 mmol). The title compound (195 mg, 97%) was obtained as a white solid.

ESI-MS Calcd for  $C_{44}H_{72}N_6O_{12}$ : 876.56. Found (m/z): 877.5 (M+H)<sup>+</sup>.

#### Example 41

##### Synthesis of Cyclo-N(Me)-O(Me)-Tyr-O-(Boc-Thr)-Ist-Hip-Leu-Pro (SAPL5).

IR (film, DCM)  $\nu$  3343, 2961, 2927, 2893, 1734, 1640, 1514, 1454, 1368, 1302, 1248, 1167, 1018  $\text{cm}^{-1}$ .

<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ 0.78 (d, J = 7.1, 3H), 0.85 (d, J = 7.0, 3H), 0.87 (d, J = 7.0, 3H), 0.89-0.93 (m, 9H), 1.10-1.20 (m, 1H), 1.20 (d, J

119

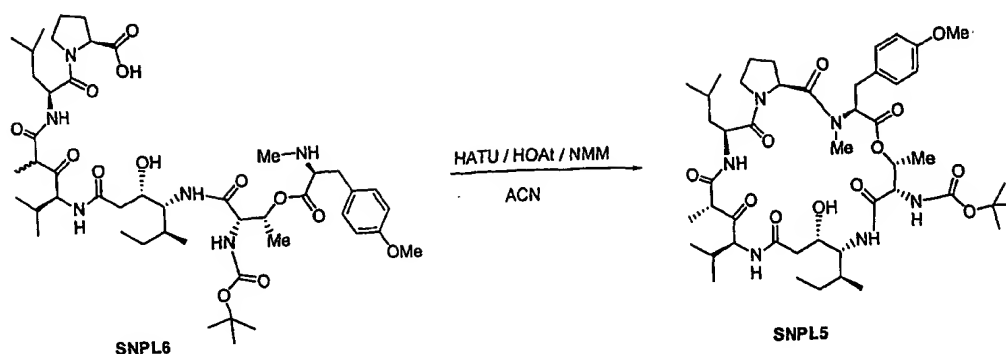
= 6.4, 3H), 1.30 (d,  $J = 6.9$ , 3H), 1.36 (m, 2H), 1.40 (m, 2H), 1.44 (s, 9H), 1.48-1.72 (m, 2H), 1.72-1.78 (m, 1H), 1.83-1.88 (m, 1H), 2.01-2.17 (m, 3H), 2.27-2.29 (m, 1H), 2.47-2.53 (m, 1H), 2.53 (s, 3H), 2.93 (bs, 1H), 3.14-3.19 (m, 2H), 3.34-3.37 (dd,  $J_1 = 14.8$ ,  $J_2 = 4.1$ , 1H), 3.54-3.56 (dd,  $J_1 = 10.5$ ,  $J_2 = 4.1$ , 1H), 3.58-3.63 (m, 1H), 3.68-3.72 (m, 1H), 3.78 (s, 3H), 3.94-3.98 (m, 1H), 3.98 (q,  $J = 7.5$ , 1H), 4.07-4.11 (3d,  $J = 3.8$ , 1H), 4.57-4.61 (m, 2H), 4.77-4.81 (m, 1H), 4.97-4.98 (q,  $J = 3.5$ , 1H), 5.02 (d,  $J = 10.5$ , 1H), 5.18 (d,  $J = 4.2$ , 1H), 6.81 (d,  $J = 8.5$ , 2H), 7.05 (d,  $J = 8.5$ , 2H), 7.19 (d,  $J = 10.2$ , 1H), 7.64 (d,  $J = 10.1$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.56, 14.68, 14.97, 15.27, 16.61, 18.45, 20.64, 23.50, 24.71, 24.78, 26.92, 27.73, 27.94, 31.55, 33.94, 33.94, 38.27, 38.52, 40.64, 46.86, 49.54, 49.65, 55.16, 55.19, 55.84, 57.12, 65.96, 67.30, 71.00, 80.27, 81.41, 114.02, 130.22, 158.53, 168.30, 169.31, 170.12, 170.29, 171.20, 172.38, 204.51.

$m/z$  (FAB) 938.9  $[(M+\text{Na})^+]$ , 55], 916.9  $[(M+\text{H})^+]$ , 100];  $m/z$  (FABHRMS) 916.532 120,  $\text{C}_{47}\text{H}_{73}\text{N}_5\text{O}_{13}$ , requires  $(M+\text{H})^+ = 916.528$  300.

#### Example 42

**Synthesis of Cyclo-N(Me)-O(Me)-Tyr-O-(Boc-Thr)-Ist-Aip-Leu-Pro (SNPL5).**



Following the procedure described for synthesis of **SAPL5**, starting from **SNPL6** (100 mg, 0.11 mmol), the title compound (40 mg, 57%) was obtained as a white solid after flash LC (silica gel, EtOAc:hex 4:1 to EtOAc neat).  $R_f=0.4$  (EtOAc).

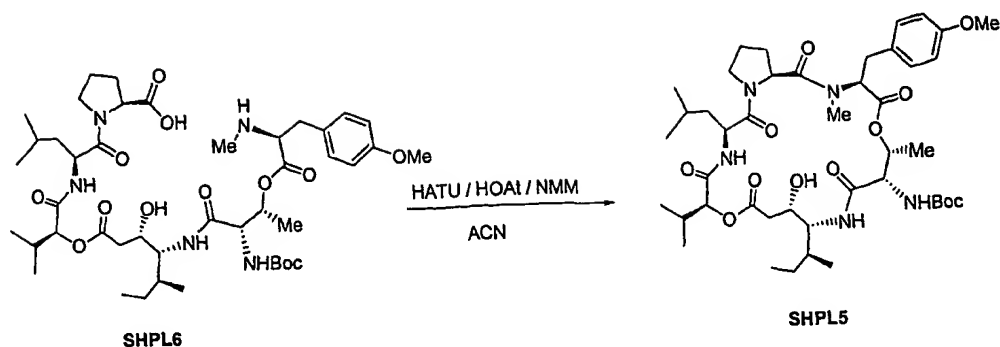
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.76 (d, J = 6.8, 3H), 0.83-0.96 (m, 15H), 1.10-1.20 (m, 1H), 1.25 (d, J = 6.4, 3H), 1.27 (d, J = 6.3, 3H), 1.32 (d, J = 6.8, 3H), 1.41 (m, 2H), 1.44 (s, 9H), 1.50-1.70 (m, 2H), 1.99-2.31 (m, 5H), 2.61 (s, 3H), 2.91-3.04 (m, 1H), 3.11-3.37 (m, 2H), 3.48-3.64 (m, 3H), 3.69-3.81 (m, 1H), 3.80 (s, 3H), 4.18 (m, 2H), 4.46-4.67 (m, 3H), 4.81 (t, J=10.7, 1H), 5.01 (m, 1H), 6.85 (d, J = 8.3, 2H), 7.07 (d, J = 8.3, 2H), 7.33 (d, J = 8.7, 1H), 7.65 (d, J = 9.2, 1H), 7.86 (d, J = 10.7, 1H).

ESI-MS Calcd for  $C_{47}H_{74}N_6O_{12}$  914.54. Found  $m/z$  915.5 ( $M+H$ )<sup>+</sup>.

### Example 43

**Synthesis of Cyclo-N(Me)-O(Me)-Tyr-O-(Boc-Thr)-Ist-Hiv-Leu-Pro (SHPL5).**

121



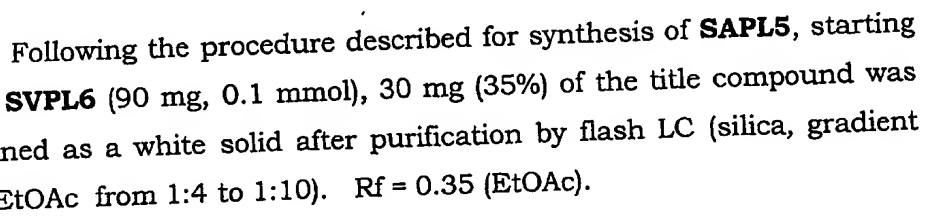
Following the procedure described for synthesis of **SAPL5**, starting from **SHPL6** (2.45 g, 2.78 mmol), the title compound (2.1 g, 88%) was obtained as a white solid after cristalization of DCM/n-heptane (1:3).  $R_f$  = 0.33 (hex-EtOAc 1:3).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-1.04 (m, 18H), 1.19 (m, 5H), 1.41 (s, 9H), 1.42 (m, 2H), 1.63 (m, 1H), 1.77 (m, 1H), 1.90 (m, 1H), 2.00-2.22 (m, 3H), 2.44 (m, 1H), 2.58 (s, 3H), 2.95 (m, 1H), 3.14 (m, 1H), 3.26 (m, 1H), 3.36 (m, 1H), 3.58 (m, 1H), 3.68 (m, 2H), 3.78 (s, 3H), 3.96 (m, 1H), 4.12 (m, 1H), 4.30 (m, 1H), 4.61 (m, 1H), 4.87 (m, 1H), 4.94 (m, 1H), 5.03 (m, 1H), 6.84 (d,  $J$  = 8.3, 2H), 7.07 (d,  $J$  = 8.3, 2H), 7.54 (d,  $J$  = 7.3, 1H), 7.69 (d,  $J$  = 6.4, 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.38, 14.57, 15.15, 18.11, 18.45, 20.67, 23.40, 24.70, 26.91, 27.89, 30.20, 33.57, 33.90, 38.51, 39.07, 46.62, 48.13, 55.16, 56.05, 56.23, 56.94, 65.67, 68.60, 71.13, 79.41, 80.01, 113.97, 129.69, 130.25, 155.75, 158.49, 168.51, 169.57, 170.24, 170.94, 171.06, 173.59.

ESI-MS: Calcd for  $\text{C}_{44}\text{H}_{69}\text{N}_5\text{O}_{12}$  859.49. Found  $m/z$  860.4 ( $\text{M}+\text{H}$ ) $^+$ .

Example 44



<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ. 0.85-1.00 (m, 18H), 1.14-1.38 (m, 8H), 1.44 (s, 9H), 1.57 (m, 2H), 1.76-1.95 (m, 2H), 2.01-2.21 (m, 2H), 2.33 (dd, J<sub>1</sub> = 7.3, J<sub>2</sub> = 14.7, 1H), 2.53 (m, 1H), 2.57 (s, 3H), 3.17 (dd, J<sub>1</sub> = 10.7, J<sub>2</sub> = 14.7, 1H), 3.35 (dd, J<sub>1</sub> = 4.4, J<sub>2</sub> = 14.2, 1H), 3.56 (dd, J<sub>1</sub> = 3.9, J<sub>2</sub> = 10.3, 1H), 3.59-3.77 (m, 4H), 3.78 (s, 3H), 4.06 (dt, J<sub>1</sub> = 3.9, J<sub>2</sub> = 9.3, 1H), 4.33 (dd, J<sub>1</sub> = 2.9, J<sub>2</sub> = 9.3, 1H), 4.38 (dd, J<sub>1</sub> = 6.8, J<sub>2</sub> = 10.3, 1H), 4.58 (dd, J<sub>1</sub> = 5.4, J<sub>2</sub> = 7.3, 1H), 4.79 (t, J = 10.3, 1H), 4.98 (d, J = 9.3, 1H), 5.03 (dd, J<sub>1</sub> = 2.4, J<sub>2</sub> = 6.3, 1H), 6.81 (bs, 1H), 6.84 (d, J = 8.3, 2H), 7.08 (d, J = 8.3, 2H), 7.24 (d, J = 9.8, 1H), 7.54 (d, J = 9.8, 1H).

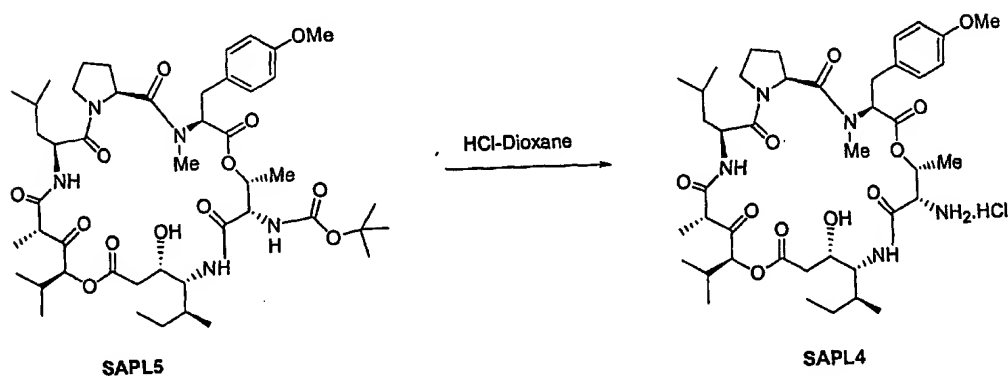
<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 11.48, 14.50, 15.20, 18.86, 19.44, 21.03, 23.64, 24.83, 24.96, 26.97, 28.01, 28.135, 30.21, 33.53, 33.96, 38.56, 38.61, 41.05, 41.66, 46.84, 48.53, 55.24, 55.51, 56.31, 57.14,

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59.75, 65.85, 70.60, 80.52, 114.11, 129.75, 130.33, 156.77, 158.67, 168.53, 170.21, 170.29, 170.73, 171.02, 174.34.

ESI-MS Calcd for  $C_{44}H_{70}N_6O_{11}$ : 858.51. Found (m/z): 859.5 (M+H)<sup>+</sup>.

## Example 45

**Synthesis of Cyclo-N(Me)-O(Me)-Tyr-O-(Thr)-Ist-Hip-Leu-Pro (SAPL4).**

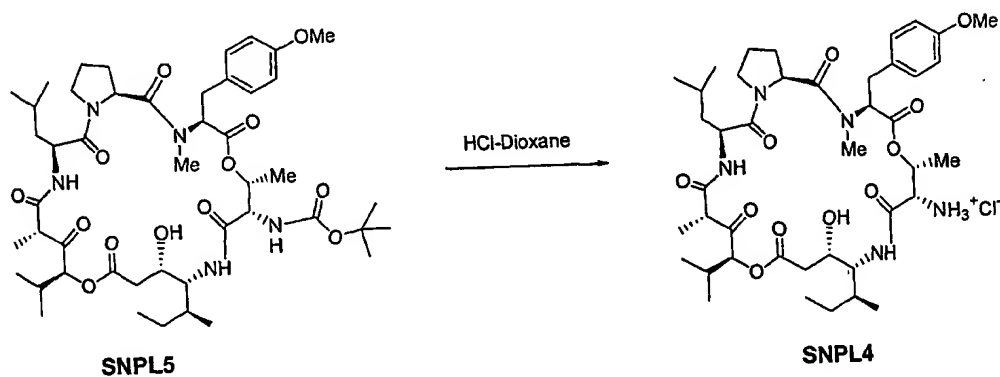
To a flask containing **SAPL5** (8.79 g, 9.6 mmol) in anh. dioxane (93 mL), a solution of hydrochloric acid in anh. dioxane (5.3 N, 122 mL, 647 mmol) was added. The resulting solution was stirred at room temperature for 8 h or until complete disappearance of the starting material. When the reaction was completed, the solution was concentrated under reduced pressure. The residue was diluted with  $CHCl_3$  (100 ml) and concentrated again. The white foam crude was coevaporated with  $CHCl_3$ /hex to give **SAPL4** (8.17 g, 100% yield) as a white solid.

m/z (FAB) 838.3 [(M+Na)<sup>+</sup>, 28], 816.3 [(M+H)<sup>+</sup>, 100].



### Example 46

### Synthesis of Cyclo-N(Me)-O(Me)-Tyr-O-(Thr)-Ist-Aip-Leu-Pro (SNPL4).



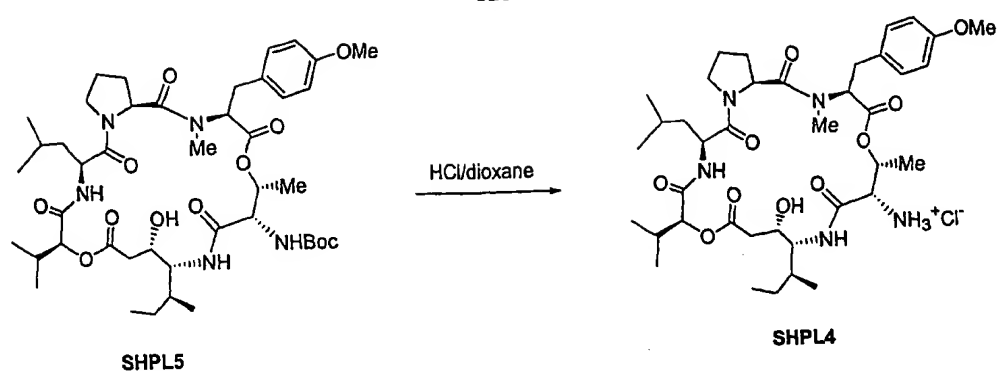
Following the procedure described for the synthesis of **SAPL4**, starting from **SNPL5** (40 mg, 43  $\mu$ mol), the title compound (36 mg, quant.) was obtained as a white solid after precipitation with Et<sub>2</sub>O.

ESI-MS: Calcd for  $C_{42}H_{65}N_5O_{11}$  815.47. Found  $m/z$  815.5 ( $M$ )<sup>+</sup>.

### Example 47

### Synthesis of Cyclo-N(Me)-O(Me)-Tyr-O-(Thr)-Ist-Hiv-Leu-Pro (SHPL4).

125

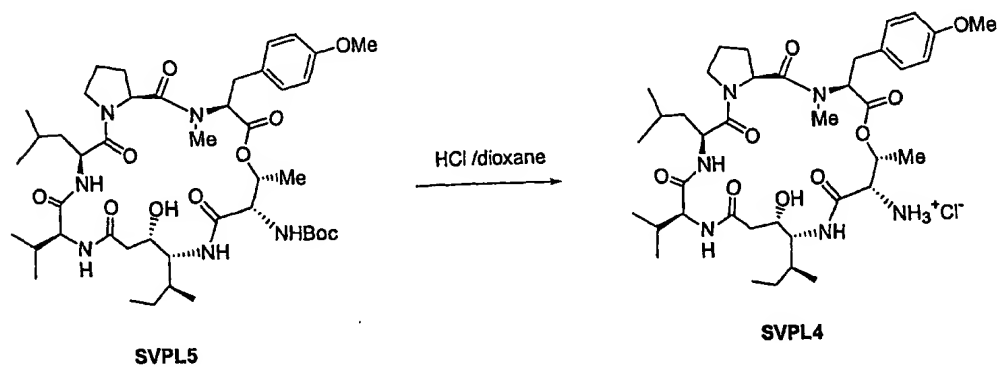


Following the procedure described for synthesis of **SAPL4**, starting from **SHPL5** (500 mg, 0.58 mmol). The title compound (440 mg, quant.) was obtained as a white solid after precipitation with Et<sub>2</sub>O.

ESI-MS: Calcd for C<sub>39</sub>H<sub>61</sub>N<sub>5</sub>O<sub>10</sub> 759.44. Found m/z 760.4 (M+H)<sup>+</sup>.

#### Example 48

#### Synthesis of Cyclo-N(Me)-O(Me)-Tyr-O-(Thr)-Ist-Val-Leu-Pro (SVPL4).



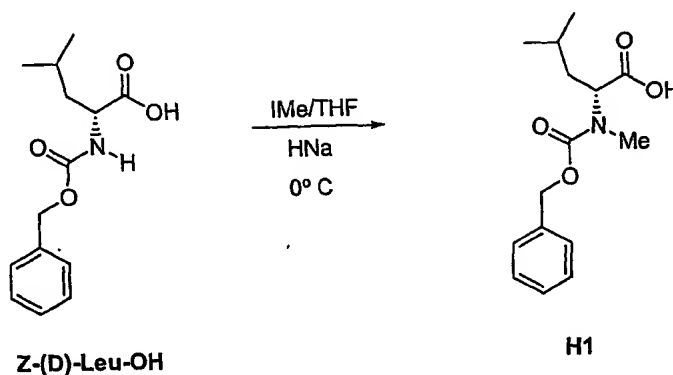
Following the procedure described for synthesis of **SAPL4**, starting from **SVPL5** (25 mg, 29 μmol). The title compound (22 mg, quant.) was obtained as a white solid after coevaporation with MTBE.

ESI-MS Calcd for  $C_{39}H_{62}N_6O_9$ : 758.5. Found (m/z): 759.5 [(M+H)]<sup>+</sup>.

#### Example 49

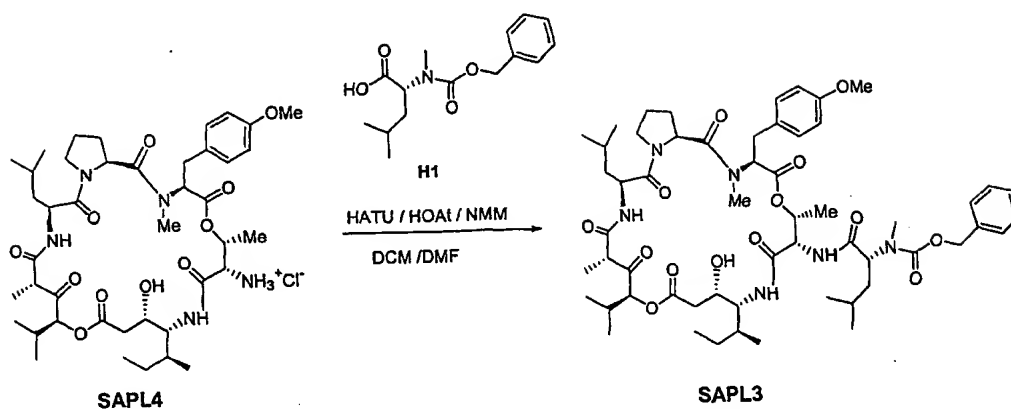
##### **Z-N-Methyl-D-Leucine (H1)**

Ref: Coggins, J. R.; Benoiton, N. L. Can. J. Chem **1971**, 49, 1968.



To a stirred solution of **Z-D-Leu-OH** (10.32 g, 38.9 mmol) in anh. THF (120 mL) at 0 °C under Ar, Iodomethane (8.55 mL, 136.1 mmol) was added dropwise by syringe. Then, sodium hydride (4.80 g, 120.6 mmol, 60% dispersion in mineral oil) was added in portions while maintaining the temperature at 0 °C. The reaction mixture was stirred at room temperature for 24 h. The solvent was eliminated under reduced pressure and the residue was dissolved in EtOAc (120 mL) and extracted with aq.  $NaHCO_3$  (300 mL, sat). The aqueous phase was washed with EtOAc (2x100 mL). The aqueous phase was cooled down, solid citric acid was added up to pH 1-2, and the solution was extracted with EtOAc (4x250 mL), dried ( $Na_2SO_4$ ), filtered and concentrated. The product was crystallized in EtOAc-Heptane (1:3) to obtain **H1** (7.84 g, 72%) as a white crystalline solid. Mp: 71-72 °C.  $[\alpha]_D^{25} +23$  (c 1, EtOH).

## Example 50

**Synthesis of Z-Didemnin A (SAPL3).**

To a flask containing HATU (8.76 g, 23.0 mmol), HOAt (3.17 g, 23.1 mmol) **SAPL4** (7.09 g, 15.8 mmol) and **H1** (3.486 g, 12.5 mmol), anh. DCM (100 mL) and anh. DMF (50 mL) were added under Ar and the solution was stirred at  $-5^{\circ}\text{C}$  (ice bath). NMM (2.3 mL, 21.0 mmol) was added dropwise by syringe, while maintaining the temperature at  $-5^{\circ}\text{C}$ . The resulting mixture reaction was stirred at  $-5^{\circ}\text{C}$  for 2 h, then allowed to reach room temperature for additional 14 h. The solvent was evaporated under reduced pressure. The crude was chased with EtOAc (100 mL) and the solution was filtered off to remove some precipitate. The solution was washed successively with aq.  $\text{KHSO}_4$  (2x100 mL, 10%), brine (100 mL), aq.  $\text{NaHCO}_3$  (100 mL, sat.) and rinse with brine (100 mL). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure to afford a yellow solid that was purified by flash LC (silica gel, gradient hex:EtOAc 2:1 to 1:1) to give **SAPL3** (7.98 g, 89% yield) as a white solid.  $R_f = 0.18$  (hex/EtOAc 1:1).

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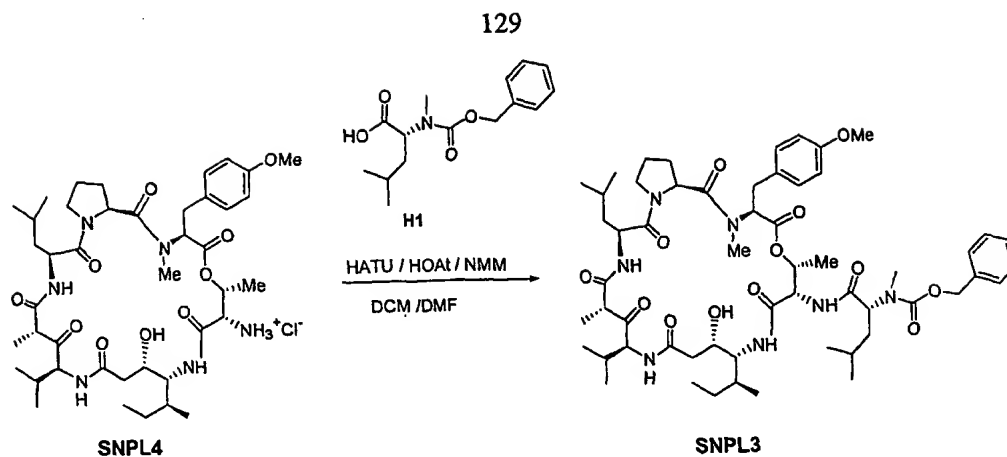
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.79-1.00 (m, 24H), 1.10-2.25 (m, 10H), 1.18 (d,  $J = 6.3$ , 3H), 1.25 (s, 3H), 1.32 (d,  $J = 6.8$ , 3H), 2.28-2.34 (m, 1H), 2.49 (dd,  $J_1=10.7$ ,  $J_2=17.0$ , 1H), 2.54 (s, 3H), 2.83 (s, 3H), 2.95 (m, 1H), 3.02-3.24 (m, 2H), 3.31-3.40 (dd,  $J_1=3.9$ ,  $J_2=14.1$ , 1H), 3.53-3.64 (m, 2H), 3.65-3.75 (m, 1H), 3.78 (s, 3H), 3.92-4.20 (m, 3H), 4.58 (t,  $J=4.8$ , 1H), 4.75-4.85 (m, 3H), 5.00 (m, 1H), 5.12-5.26 (m, 3H), 6.84 (d,  $J = 8.3$ , 2H), 6.86 (bs, 1H), 7.07 (d,  $J = 8.3$ , 2H), 7.21-7.44 (m, 6H), 7.92 (d,  $J = 8.3$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.57, 14.05, 15.18, 16.73, 18.52, 20.83, 22.62, 22.96, 23.66, 24.55, 24.81, 25.03, 25.28, 26.90, 27.86, 28.95, 31.28, 31.81, 33.91, 34.02, 38.51, 38.61, 47.03, 49.61, 55.21, 55.38, 55.50, 57.28, 66.10, 67.65, 67.93, 70.47, 81.44, 114.09, 127.83, 128.46, 129.74, 130.29, 158.60, 168.36, 169.59, 170.28, 171.20, 172.22, 204.80.

ESI-MS Calcd for  $\text{C}_{57}\text{H}_{84}\text{N}_6\text{O}_{14}$  1076.60. Found  $m/z$  1077.6 ( $\text{M}+\text{H}$ ) $^+$ .

Example 51

**Synthesis of [Aip] $^3$  Z-Didemnain A (SNPL3).**



Following the procedure described for the synthesis of **SAPL3**, starting from **SNPL4** (35mg, 41  $\mu$ mol) and **H1** (17 mg, 61  $\mu$ mol), the title compound (36 mg, 81%) was obtained as a white solid after purification by flash LC (silica gel, gradient hex-EtOAc from 1:4 to EtOAc neat).  $R_f$  = 0.30 (EtOAc).

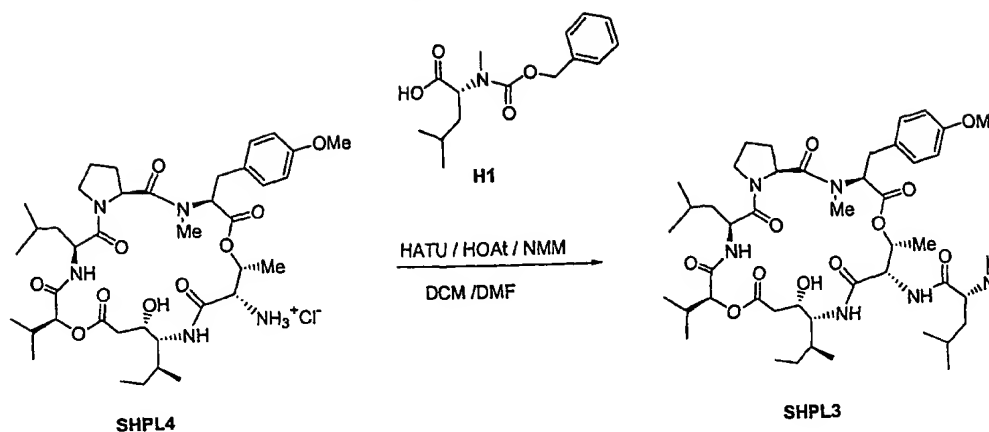
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.74 (d,  $J$  = 6.3, 3H), 0.80-1.00 (m, 21H), 1.10-2.25 (m, 10H), 1.21 (d,  $J$  = 5.8, 3H), 1.24 (s, 3H), 1.34 (d,  $J$  = 6.3, 3H), 2.61 (s, 3H), 2.86 (s, 3H), 3.12-3.25 (dd,  $J_1$ =11.2,  $J_2$ =14.1, 1H), 3.27-3.36 (dd,  $J_1$ =4.3,  $J_2$ =14.1, 1H), 3.52-3.63 (m, 3H), 3.69-3.81 (m, 2H), 3.80 (s, 3H), 4.09 (m, 3H), 4.47-4.63 (m, 3H), 4.76-4.92 (m, 2H), 5.00 (m, 1H), 5.08 (m, 1H), 5.18 (s, 2H), 6.85 (d,  $J$  = 8.3, 2H), 6.97 (d,  $J$ =6.97, 1H), 7.07 (d,  $J$  = 8.3, 2H), 7.35 (bs, 5H), 7.48 (d,  $J$  = 8.3, 1H), 7.67 (d,  $J$  = 8.3, 1H), 7.87 (d,  $J$  = 10.2, 1H).

ESI-MS Calcd for  $\text{C}_{57}\text{H}_{85}\text{N}_7\text{O}_{13}$ : 1075.62. Found  $m/z$ : 1076.6 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 52

#### Synthesis of [Hiv]<sup>3</sup> Z-Didemnins A (SHPL3)

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Following the procedure described for the synthesis of **SAPL3**, starting from **SHPL4** (116 mg, 0.15 mmol) and **H1** (63 mg, 0.23 mmol), the title compound (86 mg, 52%) was obtained as a white solid after purification by flash LC (silica gel, gradient hex-EtOAc from 1:1 to 1:2).  $R_f = 0.27$  (hex-EtOAc 1:2).

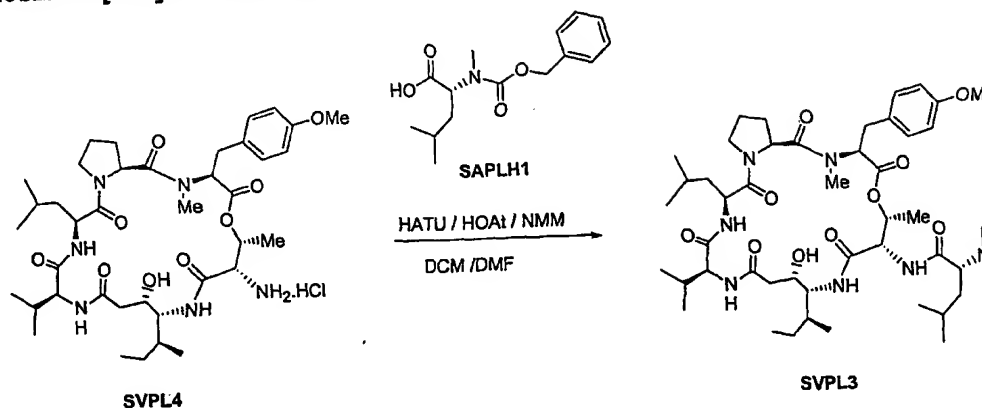
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.80-1.08 (m, 24H), 1.18 (m, 3H), 1.21 (m, 4H), 1.58 (m, 2H), 1.74 (m, 1H), 1.80-2.42 (m, 6H), 2.56 (s, 3H), 2.80 (s, 3H), 2.88 (m, 1H), 3.15 (m, 1H), 3.32 (m, 1H), 3.60 (m, 3H), 3.78 (s, 3H), 3.83 (m, 1H), 3.98 (m, 1H), 4.42 (m, 1H), 4.58 (m, 1H), 4.75 (m, 1H), 4.84 (m, 1H), 4.92 (d,  $J = 3.8$ , 1H), 5.00 (m, 1H), 5.20 (m, 2H), 6.65 (d,  $J = 6.3$ , 1H), 6.84 (d,  $J = 8.3$ , 2H), 7.07 (d,  $J = 8.3$ , 2H), 7.34 (m, 5H), 7.50 (d,  $J = 6.7$ , 1H), 7.75 (d,  $J = 7.2$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.58, 14.21, 15.39, 17.64, 18.63, 20.74, 21.88, 22.84, 23.48, 24.22, 24.75, 27.05, 27.84, 29.34, 29.99, 33.42, 33.86, 35.81, 38.52, 39.37, 46.63, 48.14, 55.13, 55.47, 55.53, 55.87, 56.92, 65.68, 67.72, 68.60, 70.61, 79.09, 113.95, 127.71, 127.86, 128.35, 129.63, 130.22, 158.48, 168.46, 169.36, 169.84, 170.29, 170.93, 171.00, 173.73.

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ESI-MS: Calcd for  $C_{54}H_{80}N_6O_{13}$  1020.58. Found  $m/z$  1021.5  
( $M+H$ )<sup>+</sup>.

## Example 53

Synthesis of [Val]<sup>3</sup> Z-Didemnin A (SVPL3)

Following the procedure described for the synthesis of **SAPL3**, starting from **SVPL4** (20 mg, 25  $\mu\text{mol}$ ) and **SAPLH1** (11 mg, 37.5  $\mu\text{mol}$ ), the title compound (19 mg, 72%) was obtained as a white solid after purification by flash LC (silica gel, gradient hex-EtOAc from 1:1 to 1:5).  $R_f$  = 0.44 (EtOAc).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.85-1.06 (m, 21 H), 1.10-1.4 (m, 5H), 1.16 (d,  $J$  = 6.6, 3H), 1.50-1.63 (m, 6 H), 1.72-1.87 (m, 2 H), 1.88-2.40 (m, 6H), 2.58 (s, 3H), 2.86 (s, 3H), 3.17 (dd,  $J_1$  = 10.5,  $J_2$  = 14.2, 1H), 3.36 (dd,  $J_1$  = 3.9,  $J_2$  = 14.2, 1H), 3.43 (bs, 1H), 3.51-3.72 (m, 4H), 3.79 (s, 3H), 3.98-4.16 (m, 1H), 4.40-4.47 (m, 2H), 4.58 (dd,  $J_1$  = 5.7,  $J_2$  = 7.8, 1H), 4.67-4.85 (m, 2H), 4.80-5.09 (m, 1H), 5.16 (d,  $J$  = 12.4, 1H), 5.24 (d,  $J$  = 12.4, 1H), 6.84 (d,  $J$  = 8.4, 1H), 6.90-6.94 (bs, 1H), 7.09 (d,  $J$  = 8.4, 1H), 7.28-7.50 (m, 6H).



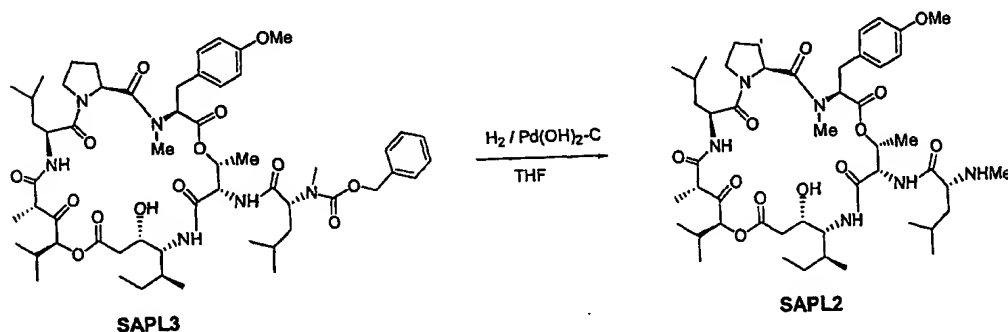
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$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.99, 14.37, 15.70, 18.55, 19.82, 21.40, 21.99, 22.81, 23.17, 23.98, 24.62, 25.00, 25.24, 27.28, 28.26, 29.93, 30.23, 33.58, 34.10, 36.16, 38.78, 41.98, 47.10, 48.79, 54.73, 55.45, 56.62, 57.40, 59.51, 66.02, 68.18, 70.37, 71.03, 114.30, 128.00, 128.27, 128.72, 129.86, 130.56, 136.49, 158.28, 158.85, 168.75, 169.27, 170.40, 170.75, 171.04, 173.91, 175.03.

ESI-MS Calcd for  $\text{C}_{54}\text{H}_{81}\text{N}_7\text{O}_{12}$ : 1019.59. Found (m/z): 1020.5 (M+H) $^+$ .

#### Example 54

#### Synthesis of Didemnin A (SAPL2)



To a solution of **SAPL3** (6.59 g, 6.1 mmol) in THF (free of stabilizer, 262 mL) degassed and purged with argon,  $\text{Pd}(\text{OH})_2\text{-C}$  (20%, 3.29 g, 50% w/w) was added. The mixture was stirred under  $\text{H}_2$  (1 atm) for 20 h, then filtered over a 0.45  $\mu\text{m}$  teflon filter and concentrated under reduced pressure to give a white solid.  $\text{CHCl}_3$  (2x25 mL) was added, and the mixture was concentrated again under reduced pressure to give **SAPL2** (5.51 g, 96%) as a white solid.  $R_f$  = 0.22 ( $\text{CHCl}_3$ :tBuOH 90:10).

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$^1\text{H}$  NMR (500 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-0.92 (m, 24H), 1.11-1.19 (m, 1H), 1.22 (d,  $J = 6.9$ , 3H), 1.32 (d,  $J = 6.8$ , 3H), 1.30-1.35 (m, 1H), 1.35-1.63 (m, 6H), 1.71-1.81 (m, 2H), 1.93-2.07 (m, 1H), 2.07-2.18 (m, 2H), 2.28-2.34 (m, 1H), 2.49-2.52 (dd,  $J_1 = 11$ ,  $J_2 = 10.5$ , 1H), 2.54 (s, 3H), 2.72 (bs, 3H), 2.79 (bs, 3H), 2.86-2.94 (bs, 1H), 2.72-2.79 (bd,  $J = 10.5$ , 1H), 3.15-3.18 (dd,  $J_1 = 14.5$ ,  $J_2 = 10.5$ , 1H), 3.33-3.36 (dd,  $J_1 = 14.5$ ,  $J_2 = 4.5$ , 1H), 3.54-3.57 (dd,  $J_1 = 10.5$ ,  $J_2 = 4.5$ , 1H), 3.56-3.61 (m, 1H), 3.78 (s, 3H), 3.96-4.00 (m, 1H), 4.03-4.08 (m, 1H), 4.11-4.80 (bs, 1H), 4.56-4.62 (m, 1H), 4.68-4.81 (m, 3H), 4.99-5.01 (q,  $J = 3.5$ , 1H), 5.16 (bs, 1H), 6.83 (d,  $J = 8.5$ , 2H), 6.95 (bs, 1H), 7.07 (d,  $J = 8.5$ , 2H), 7.21-7.25 (bs, 1H), 7.95 (bs, 1H).

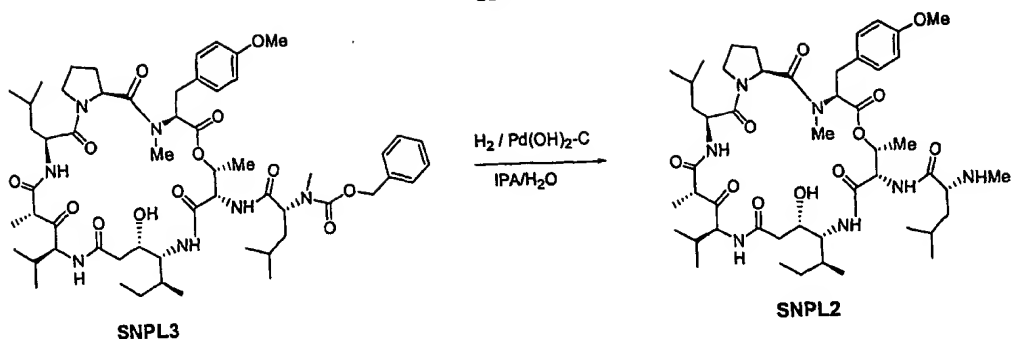
$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.55, 14.95, 15.26, 16.82, 18.56, 20.89, 22.00, 23.08, 23.76, 24.58, 24.85, 25.10, 27.12, 29.35, 29.35, 29.65, 29.69, 31.36, 33.96, 34.14, 38.51, 38.64, 40.14, 55.38, 55.56, 57.31, 66.17, 67.85, 70.58, 80.96, 80.98, 81.57, 114.12, 130.33, 158.63, 168.41, 169.33, 169.70, 170.38, 171.24, 172.28, 172.28, 172.93, 204.83.

$m/z$  (FAB) 944.2  $[(M + H)^+, 100]$ .

#### Example 55

#### Synthesis of [Aip]<sup>3</sup>-Didemnin A (SNPL2)

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To a solution of **SNPL3** (33 mg, 35  $\mu\text{mol}$ ) in a mixture of IPA/ $\text{H}_2\text{O}$  (2ml:1 ml) degassed and purged with argon,  $\text{Pd(OH)}_2\text{-C}$  (20%, 20 mg, 60% w/w) was added. The mixture was stirred under  $\text{H}_2$  (1 atm) for 20 h, then filtered over a 0.45  $\mu\text{m}$  teflon filter and concentrated under reduced pressure to give a white solid. IPA (2x5 mL) was added, and the solution was concentrated again under reduced pressure to give **SNPL2** (32 mg, 97%) as a white solid.

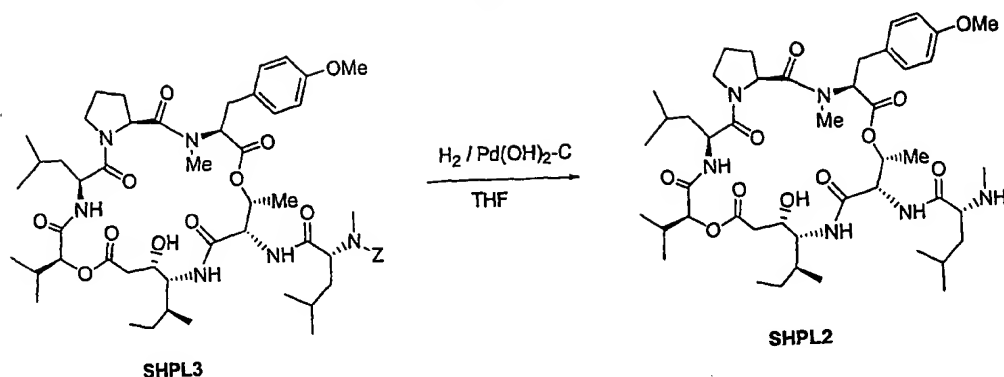
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.78 (d,  $J=6.8$ , 3H), 0.82-0.92 (m, 21H), 1.11-2.58 (m, 21H), 2.41 (s, 3H), 2.62 (s, 3H), 2.75-3.00 (m, 4H), 3.15-3.18 (dd,  $J_1 = 10.7$ ,  $J_2 = 14.7$ , 1H), 3.33-3.36 (dd,  $J_1 = 14.5$ ,  $J_2 = 4.2$ , 1H), 3.51-3.78 (m, 3H), 3.78 (s, 3H), 3.92 (m, 1H), 4.01-4.20 (m, 2H), 4.50 (t,  $J=4.8$ , 1H), 4.59 (t,  $J=6.3$ , 1H), 4.75-4.91 (m, 2H), 5.05 (m, 1H), 6.84 (d,  $J = 8.3$ , 2H), 7.07 (d,  $J = 8.3$ , 2H), 7.70 (d,  $J=5.8$ , 1H), 7.78 (d,  $J=9.7$ , 1H), 7.89 (d,  $J=6.3$ , 1H), 8.14 (d,  $J=7.8$ , 1H).

ESI-MS Calcd for  $\text{C}_{49}\text{H}_{79}\text{N}_7\text{O}_{11}$  941.58. Found  $m/z$  942.7 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 56

#### Synthesis of [Hiv]<sup>3</sup>- Didemnins A (SHPL2)

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Following the procedure described for the synthesis of **SAPL2**, starting from **SHPL3** (86 mg, 0.08 mmol), the title compound (73 mg, 97%) was obtained as a white solid.  $R_f = 0.36$  ( $\text{CHCl}_3/\text{MeOH}$  95:5).

$^1\text{H}$ NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-1.02 (m, 24H), 1.12-2.42 (m, 16H), 2.54 (s, 3H), 2.64 (s, 3H), 2.95 (m, 1H), 3.15 (m, 1H), 3.35 (m, 1H), 3.52-3.90 (m, 5H), 3.78 (s, 3H), 4.04 (m, 1H), 4.38 (m, 1H), 4.48 (m, 1H), 4.57 (m, 1H), 4.88 (m, 1H), 4.91 (d,  $J=5.3$ , 1H), 5.22 (m, 1H), 6.84 (d,  $J=8.3$ , 2H), 7.07 (d,  $J=8.3$ , 2H), 7.54 (d,  $J=9.2$ , 1H), 7.60 (d,  $J=9.4$ , 1H), 8.68 (d,  $J=6.2$ , 1H).

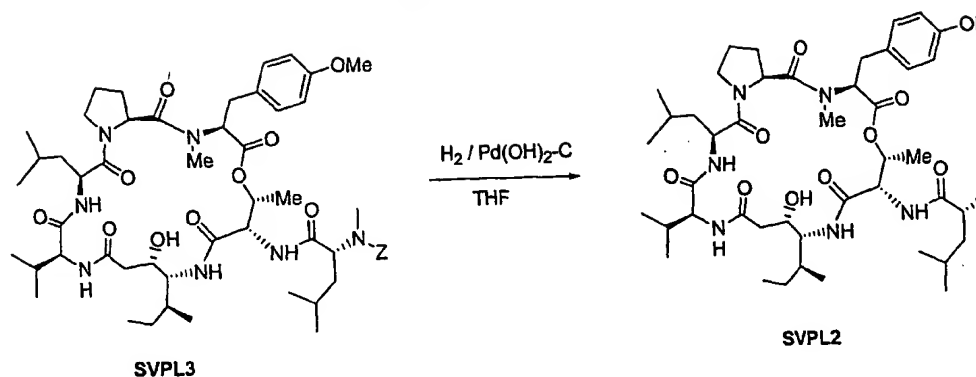
$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.78, 14.07, 16.13, 17.90, 18.56, 20.98, 21.74, 22.94, 23.60, 24.44, 24.78, 25.06, 27.17, 27.92, 30.09, 33.34, 33.89, 38.71, 40.30, 46.86, 48.22, 54.99, 55.23, 56.97, 57.21, 65.81, 68.53, 70.37, 79.47, 114.03, 129.76, 130.29, 158.57, 168.18, 169.40, 169.86, 170.20, 170.92, 174.16.

ESI-MS Calcd for  $\text{C}_{46}\text{H}_{74}\text{N}_6\text{O}_{11}$  886.54. Found  $m/z$  887.2 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 57

#### Synthesis of [Val]<sup>3</sup>- Didemnins A (SVPL2)

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Following the procedure described for the synthesis of **SAPL2**, starting from **SVPL3** (19 mg, 18  $\mu\text{mol}$ ), the title compound (16mg, 97%) was obtained as a white solid.  $R_f = 0.36$  ( $\text{CHCl}_3/\text{MeOH}$  95:5).

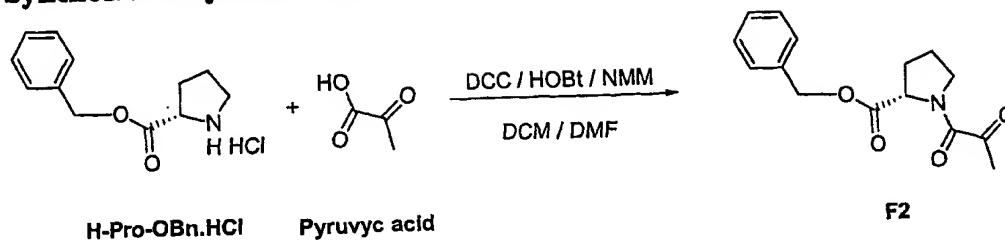
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  -0.82-1.02 (m, 21H), 1.85-1.32 (m, 9H), 1.42-1.83 (m, 7H), 1.87-2.23 (m, 8H), 2.34 (bs, 3H), 2.60 (b s, 1H), 2.85 (dd,  $J_1 = 5.7$ ,  $J_2 = 7.8$ , 1H), 3.16 (dd,  $J_1 = 10.8$ ,  $J_2 = 14.2$ , 1H), 3.37 (dd,  $J_1 = 4.2$ ,  $J_2 = 14.2$ , 1H), 3.48 (bs, 1H), 3.57-3.68 (m, 4H), 3.79 (s, 3H), 3.95-4.15 (m, 2H), 4.45-4.52 (m, 2H), 4.61 (dd,  $J_1 = 4.8$ ,  $J_2 = 7.8$ , 1H), 4.77 (t,  $J = 9.9$ , 1H), 5.02-5.15 (m, 1H), 6.84 (d,  $J = 8.4$ , 1H), 7.09 (d,  $J = 8.4$ , 1H), 7.59-7.62 (m, 2H), 7.78-7.81 (m, 2H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.98, 14.46, 16.14, 18.50, 19.83, 21.50, 22.55, 22.81, 22.95, 23.46, 23.98, 27.33, 28.27, 28.33, 29.94, 33.71, 34.16, 38.94, 41.93, 42.11, 45.72, 47.18, 48.94, 53.64, 55.03, 55.50, 56.72, 57.55, 59.22, 59.56, 66.16, 70.71, 114.36, 118.53, 120.85, 130.02, 130.58, 168.71, 169.74, 170.23, 171.18, 175.10.

ESI-MS Calcd for  $\text{C}_{46}\text{H}_{75}\text{N}_7\text{O}_{10}$ : 885.56. Found (m/z): 856.5 (M+H) $^+$ .

## Example 58

## Synthesis of Pyr-Pro-OBn (F2)



To a solution of H-Pro-OBn-HCl (10.0 g, 41.3 mmol) in anhyd DMF (50 mL) at 0 °C under Ar, NMM (4.55 mL, 43.8 mmol) was added dropwise by syringe while maintaining the temperature at 0 °C. HOBT (18.98 g, 124 mmol) was then added in portions. After 15 min, pyruvic acid (8.61 g, 97.79 mmol) dissolved in anhyd DCM (10 mL) was added dropwise by syringe while maintaining the temperature below 3 °C. Finally, DCC (22.17 g, 107.46 mmol) dissolved in DCM (80 mL) was added dropwise with a compensated funnel. The mixture was allowed to reach room temperature (2 h) and stirred for another 12 h. The reaction mixture was filtered to remove the precipitate and the solution was concentrated under reduced pressure. The residue was dissolved in EtOAc (500 mL) and washed successively with aq. KHSO<sub>4</sub> (100 mL, 10%), aq. NaHCO<sub>3</sub> (400 mL, sat.), brine (400 mL), dried (Na<sub>2</sub>SO<sub>4</sub>) and filtered. The solvent was eliminated under reduced pressure and the residue was chased with ACN (100 mL), cooled at -30 °C for 2 h and filtered to remove the excess of N,N-dicyclohexylurea. The resulting brown oil (15.82 g) was purified by flash LC (silica gel, grad. hex to hex:EtOAc 2:1) to afford **F2** (9.06 g, 66% yield) as a white solid. R<sub>f</sub> = 0.25 (hex:EtOAc 2:1).

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IR (film, DCM)  $\nu$  3035, 2956, 2884, 1744, 1717, 1645, 1499, 1443, 1383, 1352, 1273, 1175, 1092  $\text{cm}^{-1}$ .

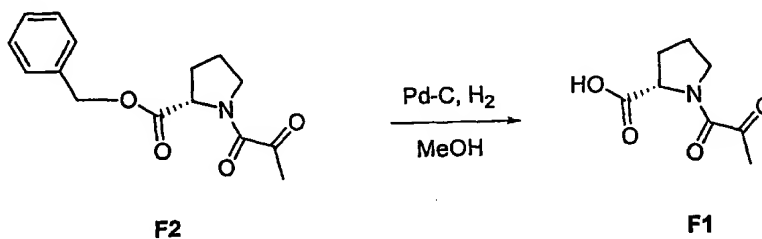
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  1.75-2.40 (m, 4H), 2.37 (s, 3H), 2.44 (s, 3H), 3.45-3.82 (m, 2H), 4.52-4.61 (m, 1H), 4.88-4.97 (m, 1H), 5.14-5.15 (m, 2H), 5.17-5.20 (m, 2H), 7.34 (bs, 5H).

$^{13}\text{C}$  NMR (50 MHz,  $\text{CDCl}_3$ )  $\delta$  22.11, 25.22, 26.5, 27.10, 28.53, 31.48, 47.53, 44.81, 59.76, 67.02, 67.31, 128.11, 128.64, 135.24, 170.1, 170.2, 198.0.

$m/z$  (CI) 293  $[(\text{M}+\text{NH}_4)^+, 100]$ . Anal. Calcd for  $\text{C}_{15}\text{H}_{17}\text{NO}_4$ : C, 65.44; H, 6.22; N, 5.08. Found: C, 65.04; H, 6.01; N, 5.11.

#### Example 59

##### Synthesis of Pyr-Pro-OH (F1)



A solution of **F2** (8.63 g, 31.34 mmol) and palladium on activated charcoal (10%, 86 mg, 10% w/w) in degassed MeOH (125 mL) was placed in a high pressure Parr reactor and purged with nitrogen gas (2x30 psi). The reaction mixture was sealed under hydrogen (30 psi) and stirred at 23°C for 4.5 h. The mixture was filtered through a 0.45  $\mu\text{m}$  teflon filter and concentrated at reduced pressure. The residue (6.29 g) was coated in  $\text{SiO}_2$  and loaded onto the top of a column (LC 5.5 x10.0 cm) and eluted

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with hex:EtOAc 1:2 to yield **F1** (4.64 g, 80%) as a white solid.  $R_f = 0.22$  (hex:EtOAc 1:1).  $[\alpha]_D^{20} -92.4$  (c 0.12,  $\text{CHCl}_3$ ). M.p. 67-69 °C.

IR (film, DCM)  $\nu$  3450-3000, 2961-2870, 1719, 1643, 1615, 1452, 1354, 1205, 1175, 1094, 1018  $\text{cm}^{-1}$ .

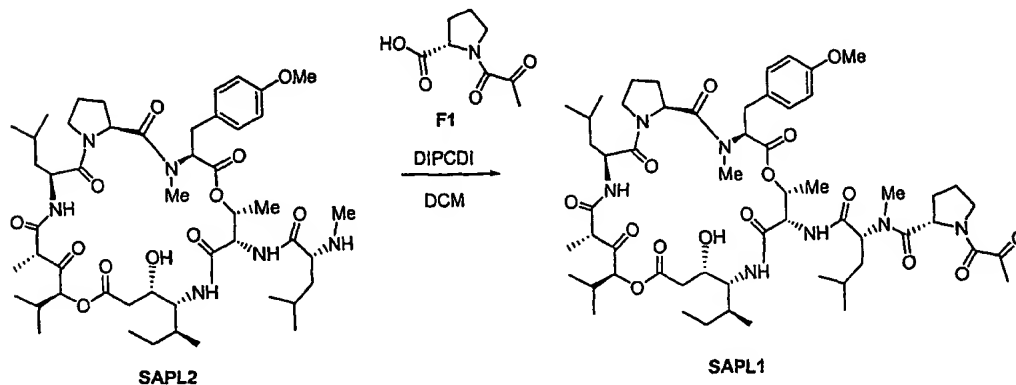
$^1\text{H}$  NMR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  1.85-2.45 (m, 4H), 2.43 and 2.47 (s, 3H), 3.42-3.85 (m, 2H), 4.52-4.61 (m, 1H), 4.88-4.97 (m, 1H), 7.21-7.40 (bs, 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  22.03, 25.23, 26.48, 27.00, 28.23, 31.44, 47.57, 48.37, 59.61, 59.39, 162.47, 162.52, 175.04, 176.29, 197.18.

$m/z$  (CI) 220  $[(\text{M}+\text{N}_2\text{H}_7)^+, 15]$ , 203  $[(\text{M}+\text{NH}_4)^+, 100]$ , 186  $[(\text{M}+\text{H})^+, 16]$ . Anal. Calcd for  $\text{C}_8\text{H}_{11}\text{NO}_4$ : C, 51.88; H, 5.99; N, 7.56. Found: C, 52.13; H, 5.85; N, 7.65

#### Example 60

#### Synthesis of Aplidine (SAPL1)





To a cold (3°C) solution of **F1** (4.908 g, 26.5 mmol) in anh. DCM (40 mL), was added, under nitrogen, a solution of DIPCDI (1.806 mg, 14.3 mmol) in DCM (10 mL) and the solution was stirred at 3°C for 60 min. Then, a solution of **SAPL2** (5.0 g, 5.3 mmol) in DCM (50 mL) was transferred *via* cannula to the previous solution under nitrogen pressure. After 90 h (4 days) at this temperature, aq HCl (50 mL, 0.1N) was added, and the reaction mixture was stirred for 15 min. Then, the organic layer was decanted and partitioned between aq. KHSO<sub>4</sub> (50 mL, 5%), aq. NaHCO<sub>3</sub> (50 mL, 5%) and brine (25 mL). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure. The resulting pale yellow solid was purified by flash LC (Lichroprep RP-18, 40-63  $\mu$ m, gradient MeOH:H<sub>2</sub>O:TFA from 70:30:0.1 to 90:10:0.1) to yield **SAPL1** (5.4 g, 93%) (mixture of rotamers).  $R_f$  = 0.40 and 0.28 (DCM:AcOEt, 2:3), 0.52 and 0.45 (CHCl<sub>3</sub>-MeOH, 9.5:0.5);  $[\alpha]_D$  -95.9 (c 1.8, CHCl<sub>3</sub>).

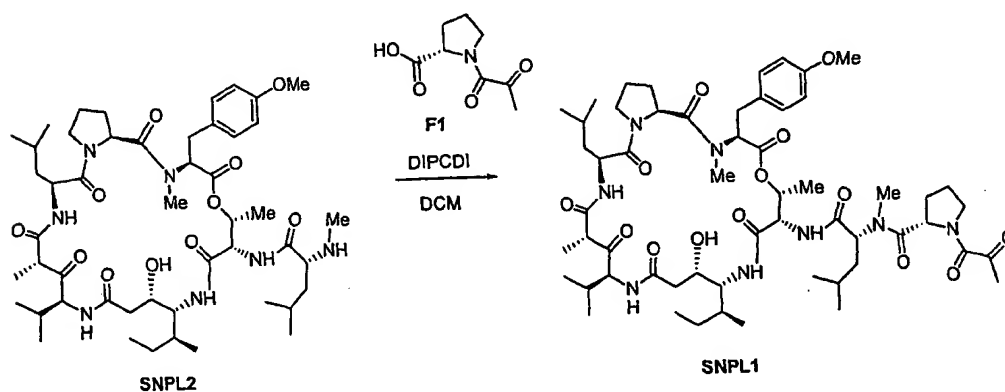
<sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>)  $\delta$  0.84-0.93 (m, 24H), 1.16-1.70 (m, 9H), 1.72-1.81 (m, 1H), 1.81-1.90 (m, 1H), 1.90-2.24 (m, 6H), 2.30-2.39 (m, 1H), 2.49 (s, 3H), 2.51 (s, 3H), 2.55 (s, 3H), 2.52-2.64 (m, 1H), 2.85 (bs, 1H), 2.94 (bs, 1H), 3.09 (s, 3H), 3.13 (s, 3H), 3.15-3.18 (m, 1H), 3.21-3.26 (dd,  $J_1$  = 15.8,  $J_2$  = 6.1, 1H), 3.32-3.36 (dd,  $J_1$  = 14.5,  $J_2$  = 4.1, 1H), 3.54-3.60 (m, 1H), 3.66-3.72 (m, 1H), 3.78 (s, 3H), 3.80-3.87 (m, 1H), 3.96-3.99 (m, 1H), 4.03-4.11 (m, 2H), 4.15-4.23 (2q,  $J$  = 7.5, 1H), 4.55-4.57 (2d,  $J_1$  = 5.5,  $J_2$  = 2.2, 1H), 4.59-4.62 (t, 1H), 4.56-4.64 (dd,  $J_1$  = 6.5,  $J_2$  = 2.5, 1H), 4.68-4.71 (t, 1H), 4.76-4.81 (t, 1H), 5.10-5.18 (m, 1H), 5.17 (d,  $J$  = 3.5, 1H), 5.18 (d,  $J$  = 3.5, 1H), 5.27-5.31 (m, 2H), 6.82 (d,  $J$  = 8.5, 2H), 6.83 (d,  $J$  = 8.5, 2H), 7.05 (d,  $J$  = 8.5, 2H), 7.06 (d,  $J$  = 8.5, 2H), 7.02 (d,  $J$  = 7.1, 1H), 7.16 (d,  $J$  = 9.5, 1H), 7.17 (d,  $J$  = 9.5, 1H), 7.59 (d,  $J$  = 5.5, 1H), 7.77 (d,  $J$  = 9.5, 1H), 7.83 (d,  $J$  = 9.4, 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.63, 11.68, 14.11, 14.70, 15.26, 15.30, 16.00, 16.20, 16.88, 16.93, 18.62, 18.85, 20.89, 20.94, 21.62, 21.36, 23.44, 23.57, 23.84, 23.93, 24.66, 24.77, 24.85, 25.02, 26.22, 26.34, 27.09, 27.6, 27.06, 27.30, 27.95, 27.99, 29.33, 29.69, 31.31-31.37, 33.97, 34.06, 36.02, 36.45, 38.68, 38.76, 41.01, 41.15, 47.00, 48.42, 48.48, 48.86, 49.20, 49.51, 54.65, 54.75, 55.26, 55.58, 55.61, 57.14, 57.27, 57.47, 57.79, 66.24, 67.80, 67.99, 70.34, 70.67, 81.0, 81.52, 114.10, 130.31, 156.0, 158.65, 161.1, 161.60, 168.20, 169.53, 169.59, 170.45, 171.25, 171.80, 171.95, 172.26, 172.33, 197.5, 204.80, 204.85.

$m/z$  (FAB) 1132.6  $[(\text{M}+\text{Na})^+, 42]$ , 1110.8  $[(\text{M}+\text{H})^+, 100]$ .

### Example 61

#### Synthesis of [Aip]<sup>3</sup>-Aplidine (SNPL1)



Following the procedure described for the synthesis of **SAPL1**, starting from **SNPL2** (10 mg, 10.6  $\mu\text{mol}$ ) and **F1** (10 mg, 54  $\mu\text{mol}$ ). The title compound (8 mg, 68%) was obtained as a white solid after

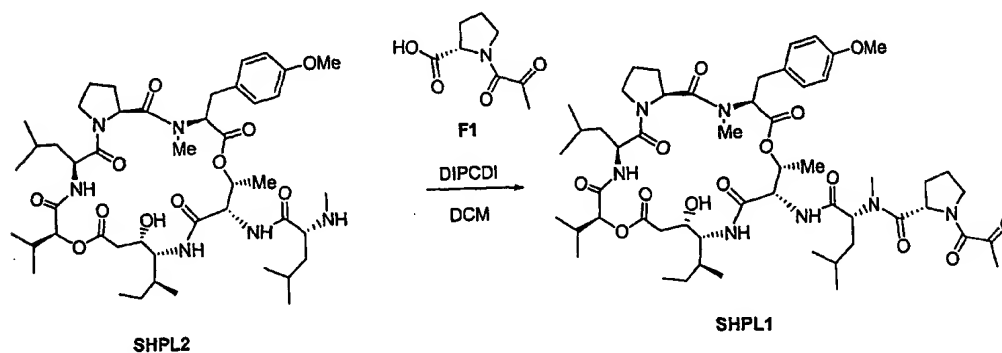
purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 10.5 and 12.0 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.80-1.03 (m, 24H), 1.11-1.70 (m, 9H), 1.72-1.81 (m, 1H), 1.81-1.90 (m, 1H), 1.90-2.24 (m, 6H), 2.30-2.39 (m, 1H), 2.53 (s, 3H) 2.55 (s, 3H), 2.65 (s, 3H), 2.52-2.66 (m, 2H), 2.94 (m, 1H), 3.07 (s, 3H), 3.11 (s, 3H), 3.15-3.18 (m, 1H), 3.22-3.31 (dd,  $J_1$  = 4.3,  $J_2$  = 15.1, 1H), 3.54-3.60 (m, 2H), 3.67-3.92 (m, 2H), 3.80 (s, 3H), 3.98 (m, 1H), 4.13-4.29 (m, 3H), 4.45-4.75 (m, 4H), 4.81 (t,  $J$  = 9.7, 1H), 5.09 (m, 1H), 5.18 (m, 1H), 5.26-5.44 (m, 3H), 6.84 (d,  $J$  = 8.3, 2H), 7.07 (d,  $J$  = 8.3, 2H), 7.30 (d,  $J$  = 8.3, 1H), 7.36 (d,  $J$  = 8.3, 1H), 7.68 (d,  $J$  = 9.7, 1H), 7.87 (d,  $J$  = 4.3, 1H), 8.09 (d,  $J$  = 9.7, 1H), 8.28 (d,  $J$  = 10.2, 1H).

ESI-MS Calcd for C<sub>57</sub>H<sub>88</sub>N<sub>8</sub>O<sub>14</sub> : 1108.64. Found (m/z): 1110.3 (M+H)<sup>+</sup>.

### Example 62

#### Synthesis of [Hiv]<sup>3</sup>-Aplidine (SHPL1)



Following the procedure described for the synthesis of **SAPL1**, starting from **SHPL2** (72 mg, 0.081 mmol) and **F1** (75 mg, 0.405 mmol).

The title compound (68 mg, 79%) was obtained as a white solid after purification by flash LC (Lichroprep RPC18, gradient ACN/H<sub>2</sub>O/TFA from 70:30:0.5 to 90:10:0.5). R<sub>f</sub>=0.49 (ACN/H<sub>2</sub>O/TFA 90:10:1).

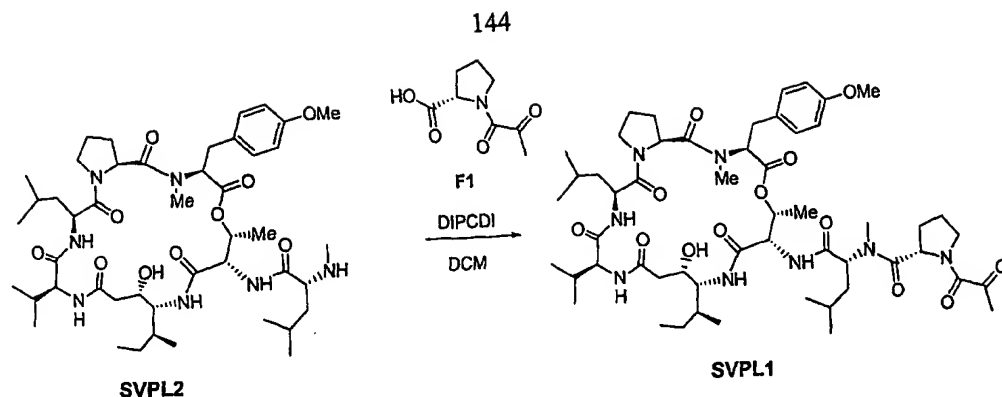
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.80-1.10 (m, 24H), 1.12-1.50 (m, 18H), 1.50-2.30 (m, 6H), 2.42 (m, 1H), 2.53 (s, 3H) 2.55 (s, 3H), 2.57 (s, 3H), 2.96-3.40 (m, 3H), 3.05 (s, 3H), 3.10 (s, 3H), 3.63 (m, 5H), 3.78 (s, 3H), 3.90 (m, 1H), 4.01 (m, 1H) 4.30 (m, 1H), 4.63 (m, 1H), 4.69 (m, 1H), 4.86 (m, 1H), 5.02 (d, J = 4.8, 1H), 5.09 (m 1H), 5.20 (m, 1H), 5.30 (m, 1H), 6.83 (d, J = 8.3, 2H), 6.89 (d, J = 6.3, 1H), 7.07 (d, J = 8.3, 2H), 7.29 (d, J = 9.7, 1H), 7.34 (m, 2H), 7.43 (d, J = 5.3, 1H), 7.74 (d, J = 9.7, 1H), 7.80 (d, J = 10.2, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 12.06, 14.22, 14.30, 16.49, 16.76, 17.84, 19.17, 21.08, 21.41, 21.54, 22.54, 23.79, 23.94, 24.05, 24.16, 24.82, 24.96, 25.05, 25.98, 26.45, 27.37, 27.57, 28.21, 28.59, 30.31, 30.83, 31.56, 31.62, 33.74, 34.24, 36.02, 36.25, 38.91, 38.96, 39.46, 39.86, 46.92, 48.44, 48.71, 49.06, 54.95, 55.49, 57.16, 57.68, 58.23, 59.13, 66.16, 66.28, 69.10, 70.83, 71.14, 79.12, 114.31, 129.96, 130.12, 130.59, 158.86, 168.69, 168.81, 169.75, 169.82, 170.18, 170.45, 170.52, 170.69, 170.84, 171.21, 171.28, 172.47, 173.17, 174.66, 174.82, 197.63, 201.38.

ESI-MS Calcd for C<sub>54</sub>H<sub>83</sub>N<sub>7</sub>O<sub>14</sub> : 1053,60. Found (m/z): 1054.9 (M+H)<sup>+</sup>.

#### Example 63

#### Synthesis of [Val]<sup>3</sup>-Aplidine (SVPL1)



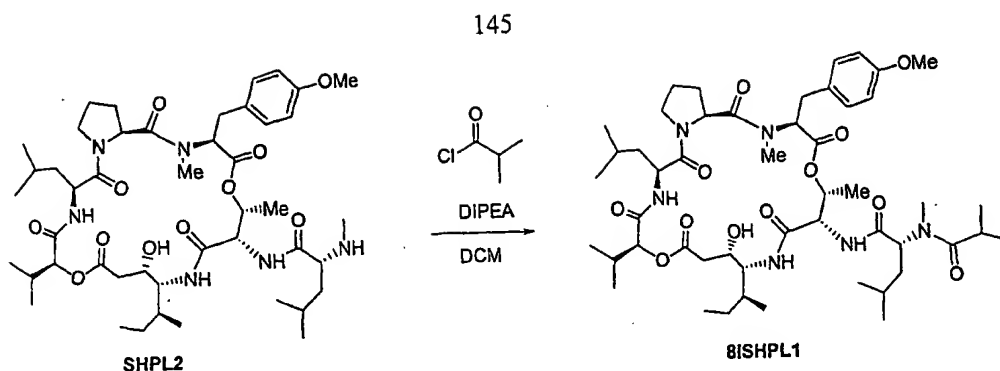
Following the procedure described for the synthesis of **SAPL1**, starting from **SVPL2** (10 mg, 11  $\mu$ mol) and **F1** (10.5 mg, 57  $\mu$ mol). The title compound (8 mg, 69%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 10.9 and 12.3 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.82-1.02 (m, 24H), 1.13-1.38 (m, 9H), 1.55 (m, 2H), 1.67-1.81 (m, 4H), 1.95-2.02 (m, 3H), 2.10-2.17 (m, 2H), 2.26-2.39 (m, 2H), 2.56 (s, 3H), 2.57 (s, 3H), 2.58 (s, 3H), 2.74-2.92 (m, 1H), 3.10 (s, 3H), 3.15 (s, 3H), 3.20 (m, 1H), 3.36 (dd,  $J_1$  = 4.4,  $J_2$  = 14.2, 1H), 3.49-3.72 (m, 5H), 3.79 (s, 3H), 3.97-4.13 (m, 2H), 4.38 (dd,  $J_1$  = 4.6,  $J_2$  = 14.2, 1H), 4.49 (m, 1H), 4.60 (m, 1H), 4.68-4.81 (m, 2H), 5.11 (m, 1H), 5.26-5.30 (m, 1H), 5.33-5.40 (m, 1H), 6.84 (d,  $J$  = 7.8, 2H), 7.08 (d,  $J$  = 8.3, 2H), 7.36-7.52 (m, 2H), 7.48 (d,  $J$  = 9.6, 1H), 7.61 (d,  $J$  = 6.8, 1H).

ESI-MS Calcd for C<sub>54</sub>H<sub>84</sub>N<sub>8</sub>O<sub>13</sub> 1052.62. Found (m/z): 1053.6 (M+H)<sup>+</sup>.

#### Example 64

#### Synthesis of [Hiv]<sup>3</sup>-[isobutyryl]<sup>8</sup>-didemnin A (8ISHPL1)



To a solution of **SHPL2** (10 mg, 11.2  $\mu\text{mol}$ ) in DCM (200  $\mu\text{l}$ ) at 0° C under Ar, was added DIPEA (3  $\mu\text{l}$ , 16.8  $\mu\text{mol}$ ) and isobutyryl chloride (1.4  $\mu\text{l}$ , 13.4  $\mu\text{mol}$ ). After 3 h at 22°C, DCM (3 ml) was added and the mixture was washed successively with aq. HCl (2 ml, 0.1N), aq. NaHCO<sub>3</sub> (2 ml, sat.) and brine (2 ml), dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated in vacuo. Purification of the residue by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 19 min) afforded the title compound (10 mg, 94%) as a white solid.

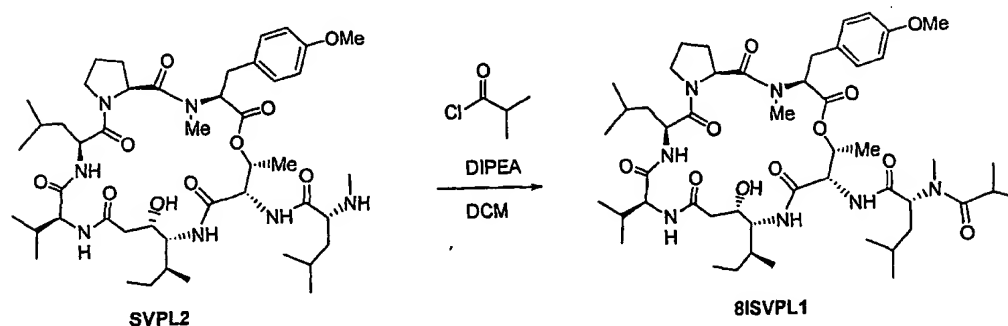
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.82-1.10 (24H, m), 1.13-1.65 (18H, m), 1.72-2.58 (6H, m), 2.56 (s, 3H), 2.89 (s, 3H), 2.92 (m, 2H), 3.13 (m, 2H), 3.36 (dd,  $J_1$  = 4.6,  $J_2$  = 15.6, 1H), 3.54-3.73 (m, 3H), 3.78 (s, 3H), 3.91 (m, 2H), 4.40 (m, 1H), 4.60 (m, 1H), 4.89 (m, 1H), 4.99 (d,  $J$  = 5.3, 1H), 5.03 (m, 1H), 5.20 (m, 1H), 6.73 (d,  $J$  = 9.3, 1H), 6.84 (d,  $J$  = 9.6, 2H), 7.08 (d,  $J$  = 9.6, 2H), 7.55 (d,  $J$  = 8.6, 1H), 7.82 (d,  $J$  = 11, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  11.78, 14.63, 15.69, 17.92, 19.03, 19.31, 19.66, 21.03, 22.29, 23.19, 23.85, 24.71, 25.14, 27.27, 28.22, 30.39, 30.51, 31.15, 33.80, 34.25, 35.61, 38.85, 39.63, 46.98, 48.51, 53.29, 53.65, 55.49, 56.01, 56.35, 57.26, 66.07, 69.07, 70.94, 79.33, 114.31, 130.08, 130.60, 158.84, 168.79, 169.75, 170.34, 170.68, 171.36, 171.73, 174.02, 179.84.

ESI-MS Calcd for  $C_{50}H_{80}N_6O_{12}$ , 956.58. Found (m/z): 957.5 (M+H)<sup>+</sup>.

### Example 65

#### Synthesis of [Val]<sup>3</sup>-[Isobutyryl]<sup>8</sup>-didemnin A (**8ISVPL1**)



Following the procedure described for the synthesis of **8ISHPL1**, starting from **SVPL2** (20 mg, 22.6  $\mu$ mol). The title compound (19 mg, 88%) was obtained after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15, flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 19 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  0.81-1.02 (m, 24 H), 1.14-1.38 (m, 5H), 1.15 (d, J = 6.6, 3H), 1.19 (d, J = 6.6, 3H), 1.38-1.80 (m, 7H), 1.80-2.40 (m, 6 H), 2.57 (s, 3H), 2.58-2.64 (m, 1H), 2.85-2.92 (m, 1H), 2.93 (s, 3H), 3.16 (dd, J<sub>1</sub> = 10.5, J<sub>2</sub>=14.4, 1H), 3.36 (dd, J<sub>1</sub> = 4.5, J<sub>2</sub>=14.4, 1H), 3.39 (bs, 1H), 3.56 (dd, 1H, J = 4.5, 10.8), 3.59-3.72 (m, 3H), 3.78 (s, 3H), 4.01 (td, J<sub>1</sub> = 3.3, J<sub>2</sub>=10.2, 1H), 4.39-4.47 (m, 2H), 4.58 (dd, J<sub>1</sub> = 5.7, J<sub>2</sub>=7.5, 1H), 4.79 (t, J = 9.9, 1H), 5.03-5.14 (m, 2H), 6.84 (d, J = 8.4,

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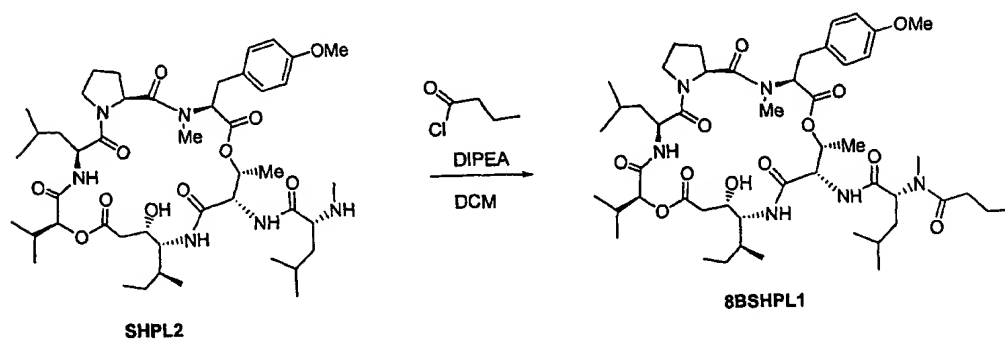
2H), 7.04 (d, J = 7.8, 1H), 7.08 (d, J = 8.4, 1H), 7.36 (d, J = 9.0, 1H), 7.45 (d, J = 10.2, 1H), 7.51 (d, J = 10.2, 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  180.24, 175.10, 173.18, 171.14, 170.72, 170.42, 169.38, 168.73, 158.87, 130.60, 130.02, 114.33, 71.31, 70.50, 66.11, 59.47, 57.42, 56.56, 55.51, 54.93, 53.84, 48.83, 47.13, 41.94, 38.94, 35.67, 34.17, 33.63, 31.32, 31.10, 29.96, 28.32, 27.25, 25.30, 25.05, 24.82, 24.01, 23.22, 21.42, 19.88, 19.72, 11.25, 18.51, 15.60, 14.32, 11.96.

ESI-MS Calcd for  $\text{C}_{50}\text{H}_{81}\text{N}_7\text{O}_{11}$ : 955.60. Found 956.8 (M+H) $^+$ .

#### Example 66

#### Synthesis of [Hiv] $^3$ -[Butyryl] $^8$ -didemnin A (8BSHPL1)



Following the procedure described for the synthesis of **8ISHPL1**, starting from **SHPL2** (10 mg, 11.2  $\mu\text{mol}$ ). The title compound (9 mg, 84%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/ $\text{H}_2\text{O}$  85:15, flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 18.6 min).



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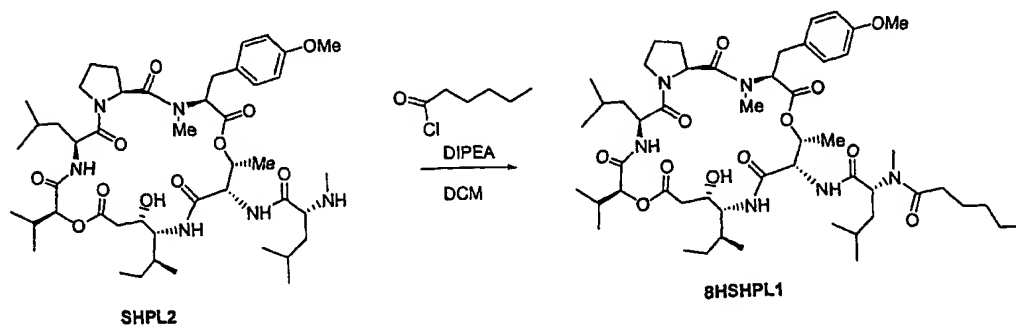
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-1.10 (m, 24H), 1.11-1.72 (m, 18H), 1.75-2.51 (m, 6H), 2.56 (s, 3H), 2.84 (s, 3H), 2.92 (m, 2H), 3.15 (m, 2H), 3.35 (dd,  $J_1 = 5.0$ ,  $J_2 = 15.6$ , 1H), 3.54-3.77 (m, 3H), 3.79 (s, 3H), 3.91 (m, 2H), 4.40 (m, 1H), 4.60 (m, 1H), 4.89 (m, 1H), 4.98 (d,  $J_1 = 6.0$ , 1H), 5.04 (m, 1H), 5.20 (m, 1H), 6.78 (d,  $J_1 = 9.6$ , 1H), 6.84 (d,  $J_1 = 9.6$ , 2H), 7.08 (d,  $J_1 = 9.6$ , 2H), 7.54 (d,  $J_1 = 9.6$ , 1H), 7.82 (d,  $J_1 = 10.6$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.82, 14.15, 14.55, 15.72, 17.96, 18.67, 19.01, 21.05, 22.37, 23.13, 23.84, 24.71, 25.14, 27.30, 28.21, 29.92, 30.38, 30.63, 33.78, 34.25, 35.69, 35.97, 38.85, 39.61, 46.98, 48.52, 53.27, 55.49, 55.99, 56.25, 57.27, 66.07, 69.07, 70.96, 79.40, 114.31, 130.08, 130.59, 158.85, 168.78, 169.73, 170.42, 170.64, 171.36, 171.65, 174.07, 175.79.

ESI-MS Calcd for  $\text{C}_{50}\text{H}_{80}\text{N}_6\text{O}_{12}$ : 956,60. Found (m/z): 957.8 (M+H) $^+$ .

#### Example 67

#### Synthesis of [Hiv] $^3$ -[hexanoyl] $^8$ -didemnin A (8HSHPL1)



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Following the procedure described for the synthesis of **8ISHPL1**, starting from **SHPL2** (10 mg, 11.2  $\mu$ mol), the title compound (9 mg, 82%) was obtained as a white solid, after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15, flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 27.8 min).

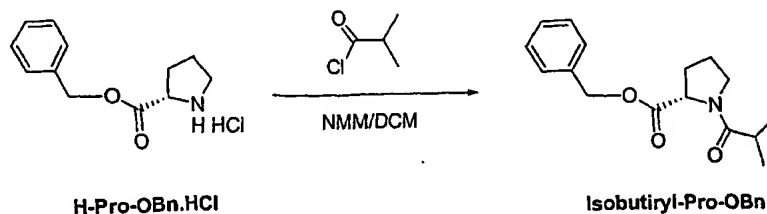
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.82-1.10 (m, 24H), 1.11-1.72 (m, 22H), 1.80-2.51 (m, 6H), 2.56 (s, 3H), 2.84 (s, 3H), 2.93 (m, 2H), 3.14 (m, 2H), 3.35 (dd,  $J_1$  = 4.4,  $J_2$  = 14.1, 1H), 3.54-3.76 (m, 3H), 3.79 (s, 3H), 3.91 (m, 2H), 4.41 (m, 1H), 4.60 (m, 1H), 4.88 (m, 1H), 4.98 (d,  $J_1$  = 5.3, 1H), 5.03 (m, 1H), 5.19 (m, 1H), 6.76 (d,  $J_1$  = 8.7, 1H), 6.84 (d,  $J_1$  = 8.7, 2H), 7.08 (d,  $J_1$  = 8.7, 2H), 7.51 (d,  $J_1$  = 8.8, 1H), 7.81 (d,  $J_1$  = 9.7, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  11.59, 13.94, 14.34, 15.49, 17.73, 18.77, 20.81, 22.14, 22.46, 22.90, 23.60, 24.47, 24.69, 24.90, 27.08, 27.97, 30.14, 30.40, 31.54, 33.53, 33.76, 34.02, 35.47, 38.62, 39.40, 46.76, 48.28, 53.04, 55.26, 55.78, 55.90, 57.04, 65.84, 68.82, 70.72, 79.17, 114.07, 129.84, 130.36, 158.61, 168.54, 169.51, 170.15, 170.41, 171.12, 171.42, 173.86, 175.75.

ESI-MS Calcd for C<sub>52</sub>H<sub>84</sub>N<sub>6</sub>O<sub>12</sub>: 984,61. Found (m/z): 985.8 (M+H)<sup>+</sup>.

#### Example 68

##### Synthesis of Isobutyryl-Pro-OBn

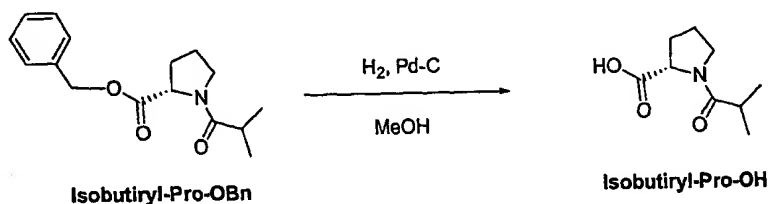


To a solution of H-Pro-OBn.HCl (500 mg, 2.07 mmol) in DCM (10 ml) at 0° C, NMM (680  $\mu$ l, 6.21 mmol) was added under argon. After 10 min, isobutyryl chloride (240  $\mu$ l, 2.27 mmol) was added and the reaction mixture was allowed to warm to 20° C and stirred for 5 h. The mixture was filtered and the filtrate washed successively with aq. HCl (15 ml, 1N), aq NaHCO<sub>3</sub> (10 ml, sat.), and brine (10 ml), then dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure to afford 560 mg (98%) of the title compound.  $R_f$  = 0.42 (hex:EtOAc 1:1).

<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.20-1.40 (2d, 6H), 1.90-2.35 (m, 4H), 2.35 (q, 1H), 3.40-3.80 (m, 2H), 4.30 [m, 1H], 5.20 (m, 2H), 7.40 (m, 5H).

#### Example 69

#### Synthesis of Isobutyryl-Pro-OH



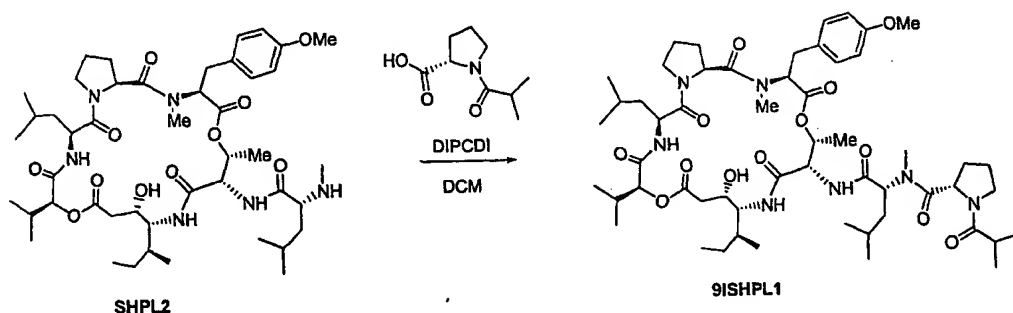
To a solution of Isobutyryl-Pro-OBn (430 mg, 1.56 mmol) in degassed MeOH was added Pd/C (10%) (43 mg, 10% w/w) and then flushed successively with Ar and bubbled with hydrogen. The mixture was stirred under H<sub>2</sub> for 14 h and then degassed and filtered. The solution was concentrated and the residue crystallized with MTBE/hex to give 140 mg (48%) of the title compound as a white solid.

<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.20 (m, 6H), 1.90-2.10 (m, 3H), 2.50 (m, 1H), 2.70 (q, 1H), 3.40-3.70 (m, 2H), 4.60 (dd, 1H).

ESI-MS Calcd for  $C_9H_{15}NO_3$ : 185.11. Found (m/z): 186.1 (M+H)<sup>+</sup>.

### Example 70

#### Synthesis of [Hiv]<sup>3</sup>-[Isobutyryl]<sup>9</sup>-aplidine (**9ISHPL1**)



To a solution of Isobutyryl-Pro-OH (10 mg, 54  $\mu$ mol) in DCM (150  $\mu$ l) at 0° C, was added DIPCPI (5  $\mu$ l, 32  $\mu$ mol). Stirring was continued for 60 min and then, the mixture was transferred to a flask containing **SHPL2** (10 mg, 11.2  $\mu$ mol) in DCM (150  $\mu$ l). After 4d at 2-4°C the mixture was diluted with DCM (2 ml) and washed successively with aq HCl (1 ml, 0.1 N), aq. NaHCO<sub>3</sub> (1 ml, sat.) and brine (1 ml), the organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The residue was purified by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm, t<sub>R</sub> = 13 and 14 min) to afford **9ISHPL1** (9 mg, 72%) as a white solid.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.82-1.10 (m, 24H), 1.12-1.50 (m, 18H), 1.52-2.70 (m, 10H), 2.56 (s, 3H), 3.00-3.44 (m, 4H), 3.08 (s, 3H), 3.55-3.72 (m, 5H) 3.78 (s, 3H), 4.00 (m, 3H), 4.26 (m, 1H), 4.62 (m, 2H), 4.86 (m, 1H), 5.02 (d, J= 5.3, 1H), 5.30 (m, 2H), 6.84 (d, J= 9.0, 2H), 7.07

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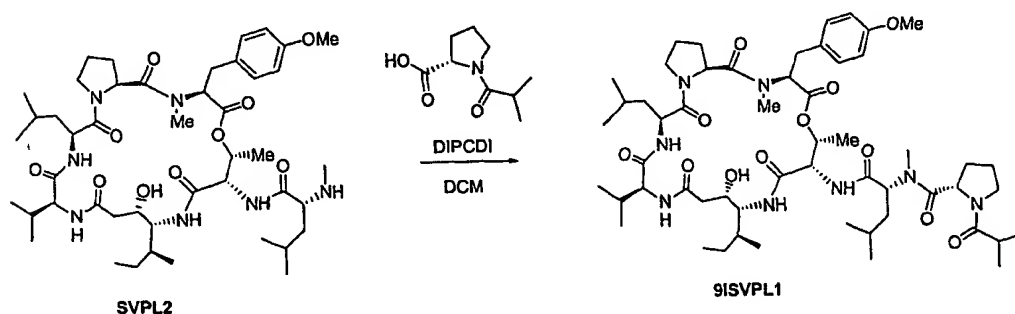
(d, J= 9.0, 2H), 7.29 (d, J= 11.0, 1H), 7.80 (d, J= 9.0, 1H); 7.88 (d, J= 11, 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  12.04, 14.44, 16.91, 17.94, 18.96, 19.03, 19.15, 21.07, 21.59, 23.87, 24.10, 24.89, 25.08, 26.04, 27.54, 28.21, 28.87, 30.33, 31.61, 32.64, 33.77, 34.24, 36.19, 39.07, 39.33, 39.81, 46.87, 47.52, 48.45, 54.74, 55.49, 56.20, 57.08, 58.50, 66.41, 69.13, 71.48, 79.16, 114.27, 130.30, 130.59, 158.80, 168.55, 169.88, 170.86, 171.06, 171.22, 173.63, 174.92, 176.19.

ESI-MS Calcd for  $\text{C}_{55}\text{H}_{87}\text{N}_7\text{O}_{13}$  : 1053.64. Found (m/z): 1054.6 (M+H) $^+$ .

#### Example 71

#### Synthesis of [Val] $^3$ -[Isobutyryl] $^9$ -aplidine (9ISVPL1)



Following the procedure described for the synthesis of **9ISHPL1**, starting from **SVPL2** (10 mg, 11.2  $\mu\text{mol}$ ), the title compound (9 mg, 77%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/ $\text{H}_2\text{O}$  85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 15.3 min).

153

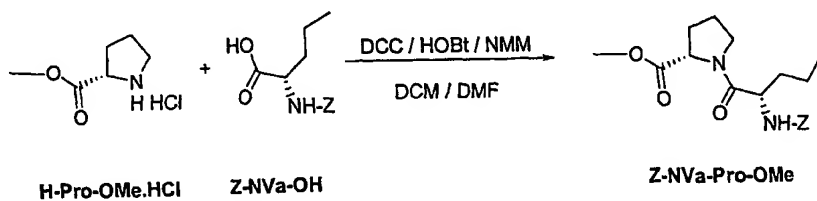
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.82-1.01 (m, 24H), 1.14-1.37 (m, 12H), 1.148-2.35 (m 8H), 2.55 (s, 3H), 2.57-2.68 (m, 1H), 2.77-2.83 (m, 1H), 3.12 (s, 3H), 3.19-3.23 (m, 1H), 3.35-3.41 (m, 2H), 3.52-3.56 (m, 1H), 3.58-3.70 (m, 3H), 3.78 (s, 3H), 4.03-4.13 (m, 1H), 4.33-4.35 (m, 1H), 4.44-4.48 (m, 1H), 4.55-4.66 (m, 2H), 4.70-4.83 (m, 1H), 5.35-5.39 (m, 2H), 6.82 (d,  $J = 8.4$ , 2H), 7.06 (d,  $J = 8.4$ , 2H), 7.25 (bs, 2H), 7.37 (d, 10.5, 1H), 7.46 (d,  $J = 8.7$ , 1H), 7.60 (d,  $J = 9.3$ , 1H), 8.07 (bs, 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  12.10, 14.44, 16.84, 18.63, 18.95, 19.92, 21.31, 21.61, 23.91, 24.05, 24.86, 25.00, 25.30, 26.06, 27.41, 28.33, 28.93, 30.00, 31.76, 32.65, 33.59, 31.16, 36.21, 39.17, 41.71, 42.19, 47.08, 47.57, 48.88, 54.37, 54.65, 55.50, 56.15, 57.33, 58.70, 59.33, 66.45, 70.92, 71.54, 114.29, 130.28, 130.59, 158.82, 168.41, 170.08, 170.52, 170.69, 171.03, 172.65, 173.80, 175.68, 176.42.

ESI-MS Calcd for  $\text{C}_{55}\text{H}_{88}\text{N}_8\text{O}_{12}$  : 1052.65. Found ( $m/z$ ): 1053.8 ( $\text{M}+\text{H}$ ) $^+$ .

### Example 72

#### Synthesis of Z-NVa-Pro-OMe



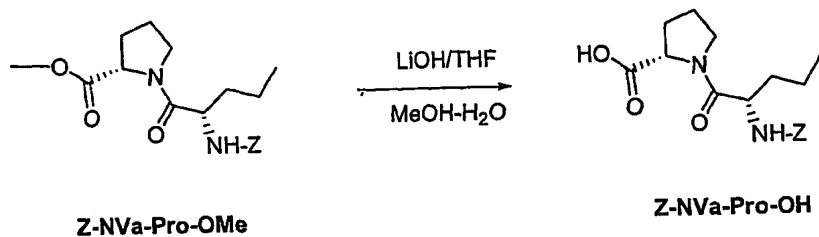
Following the procedure described for the synthesis of **F2**, from Z-NVa-OH (261 mg, 1.04 mmol), H-Pro-OMe.HCl (156.6 mg, 0.94 mmol), the title compound (315 mg, 87%) was obtained as a colourless oil after

purification by LC-silica (hex-EtOAc, gradient 3:1 to 1:1).  $R_f$  0.42 (hex-EtOAc 1:1).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.97 (t,  $J$  = 6.9, 2H), 1.44 (six,  $J$  = 7.5, 2H), 1.53-1.65 (m, 1H), 1.68-1.77 (m, 1H), 1.82-2.09 (m, 3H), 2.17-2.24 (m, 1H), 3.43-3.80 (m, 2H), 3.71 (s, 3H), 4.45-4.55 (m, 2H), 5.07 (s, 2H), 5.51 (d,  $J$  = 8.4, 1H), 7.32-7.35 (m, 5H).

### Example 73

#### Synthesis of NVa-Pro-OH



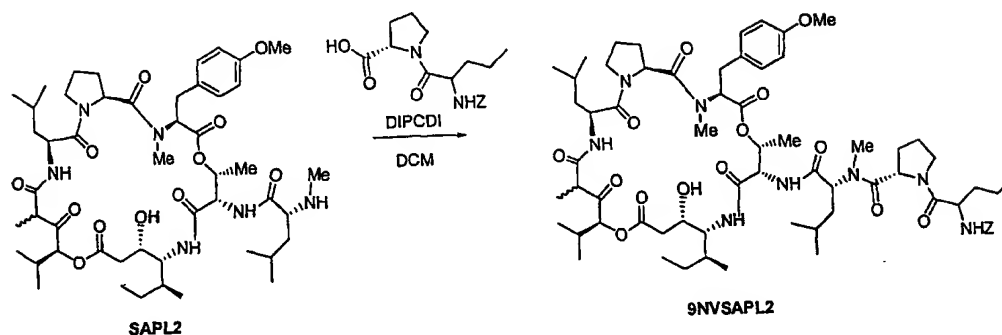
To a solution of Z-NVa-Pro-OMe (36 mg, 99  $\mu\text{mol}$ ) in a mixture of THF and MeOH (130  $\mu\text{l}$ :130  $\mu\text{l}$ ) at  $0^\circ\text{C}$ , aq LiOH (130  $\mu\text{l}$ , 15% w/w) was added. After 6 h of stirring the reaction mixture was partitioned between  $\text{H}_2\text{O}$  (3 ml) and diethyl ether (3x2 ml). The organic phase was then extracted with  $\text{NaHCO}_3$  (3x 2 ml). The combined aqueous phases were neutralized (pH= 5) with aq. HCl (0.1 N) and partitioned with  $\text{Et}_2\text{O}$  (3x3 ml). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated to give the title compound (36 mg, quant) as a colourless oil.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.96 (t,  $J$  = 6.9, 2H), 1.41 (six,  $J$  = 7.4, 2H), 1.53-1.65-1.77 (m, 2H), 1.82-2.10 (m, 3H), 2.17-2.24 (m, 1H), 3.52-3.81 (m, 2H), 4.45-4.58 (m, 2H), 5.07 (bs, 2H), 5.81 (d,  $J$  = 8.4, 1H), 7.30-7.35 (m, 5H), 7.41 (bs, 1H).

ESI-MS Calcd for  $C_{18}H_{24}N_2O_5$ : 348.17. Found (m/z): 349.2 (M+H)<sup>+</sup>.

#### Example 74

#### Synthesis of [ZNVa-Pro]<sup>9</sup>-aplidine (9NVSAPL2)



Following the procedure described for the synthesis of **SAPL1**, starting from **SAPL2** (18 mg, 19  $\mu$ mol) and Z-NVa-Pro-OH (34 mg, 97  $\mu$ mol), the title compound (16 mg, 66%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_r$  = 29 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  0.84-0.96 (m, 27H), 1.12-1.85 (m, 19H), 2.00-2.25 (m, 7H), 2.30-2.40 (m, 1H), 2.54 (s, 3H), 2.62 (dd,  $J_1$  = 10.5,  $J_2$  = 17.7, 1H), 2.93 (d, 4.2, 1H), 3.14 (s, 3H), 3.14-3.20 (m, 1H), 3.28-3.34 (m, 2H), 3.50-3.67 (m, 4H), 3.77-3.80 (m, 1H), 3.79 (s, 3H), 3.82-3.91 (m, 1H), 4.00-4.17 (m, 2H), 4.27 (dd,  $J_1$  = 6.3,  $J_2$  = 13.2, 1H), 4.43-4.51 (m, 2H), 4.58-4.63 (m, 1H), 4.69-4.75 (m, 1H), 4.77-4.82 (m, 1H), 5.07 (d, 1H,  $J$  = 12.9, 2H), 5.13 (d,  $J$  = 12.9, 1H), 5.32-5.41 (m, 2H), 6.07 (d,  $J$  = 8.7, 1H), 6.83 (d,  $J$  = 8.4, 2H), 7.06 (d,  $J$  = 8.4, 2H), 7.17 (d,  $J$  = 9.9, 1H), 7.32 (m, 5H), 7.83 (d,  $J$  = 9.0, 1H).



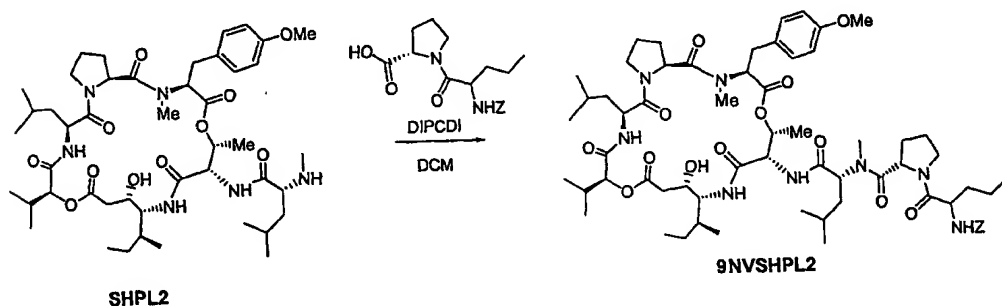
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$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.68, 13.74, 14.56, 15.24, 16.39, 16.85, 18.61, 20.94, 21.26, 23.34, 23.70, 24.76, 24.90, 25.02, 26.00, 27.17, 27.81, 28.54, 31.29, 31.40, 33.30, 33.85, 33.86, 36.19, 38.62, 38.84, 41.31, 46.91, 47.21, 49.38, 49.49, 52.50, 54.96, 55.24, 55.25, 56.50, 57.17, 57.96, 62.53, 66.40, 67.93, 70.64, 81.37, 114.04, 127.89, 127.77, 128.32, 129.97, 130.29, 136.84, 156.72, 158.55, 168.48, 169.36, 169.58, 170.52, 171.27, 171.71, 172.54, 173.22, 205.08.

ESI-MS Calcd for  $\text{C}_{67}\text{H}_{100}\text{N}_8\text{O}_{16}$ : 1273.7. Found (m/z): 1273.7 (M+H) $^+$ .

#### Example 75

#### Synthesis of [Hiv] $^3$ -[Z-NVa-Pro] $^9$ -aplidine (9NVSHPL2)



Following the procedure described for the synthesis of **SAPL1**, starting from **SHPL2** (10 mg, 11.2  $\mu\text{mol}$ ), and Z-NVa-Pro-OH (20 mg, 56  $\mu\text{mol}$ ), the title compound (8 mg, 60%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/ $\text{H}_2\text{O}$  85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 26.7 min).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-1.10 (m, 24H), 1.12-1.80 (m, 22H), 1.82-2.35 (m, 6H), 2.42 (m, 1H), 2.56 (s, 3H), 2.96-3.38 (m, 4H),

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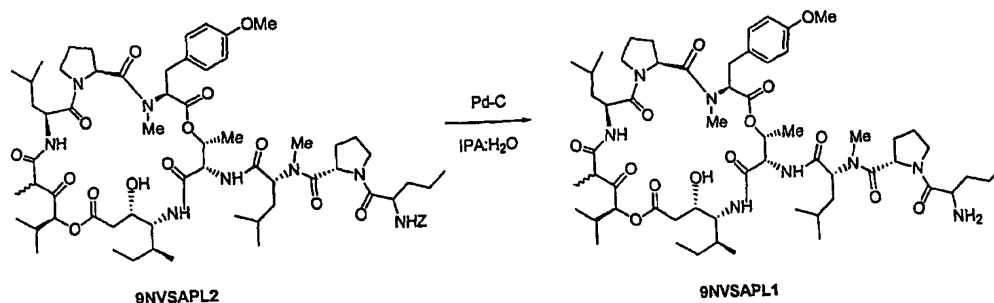
3.10 (s, 3H), 3.48-3.72 (m, 5H), 3.78 (s, 3H), 3.88 (m, 1H), 4.01 (m, 1H), 4.18 (m, 1H), 4.47 (m, 1H), 4.68 (m, 2H), 4.87 (m, 1H), 5.02 (d,  $J_1 = 5.3$ , 1H), 5.08 (m, 2H), 5.28 (m, 1H), 5.42 (m, 1H), 6.10 (d,  $J = 8.3$ , 1H), 6.84 (d,  $J = 8.3$ , 2H), 7.07 (d,  $J = 8.3$ , 2H), 7.31 (m, 6H), 7.72 (d,  $J = 4.3$ , 1H), 7.78 (d,  $J = 8.7$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  12.06, 13.88, 14.22, 16.85, 17.76, 18.89, 19.18, 21.12, 21.47, 23.72, 23.98, 25.08, 26.25, 27.57, 28.15, 28.79, 30.32, 31.61, 33.45, 33.72, 34.07, 35.95, 38.92, 39.59, 39.90, 46.87, 47.44, 48.46, 52.76, 55.21, 55.49, 56.77, 57.17, 58.37, 66.41, 66.65, 69.22, 71.14, 79.07, 114.24, 127.87, 128.00, 128.56, 130.25, 130.55, 157.00, 158.79, 168.82, 169.86, 170.36, 170.58, 170.77, 171.30, 171.86, 173.28, 174.94.

ESI-MS Calcd for  $\text{C}_{64}\text{H}_{96}\text{N}_8\text{O}_{15}$  1216.7. Found  $m/z$ : 1217.5 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 76

#### Synthesis of [NVa-Pro] $^9$ -aplidine (9NVSAPL1)



A degassed mixture of **9NVSAPL2** (10 mg, 7.8  $\mu\text{mol}$ ) and Pd/C (10%, 5 mg) in IPA: $\text{H}_2\text{O}$  (0.2 ml:0.1 ml), was saturated (and maintained at

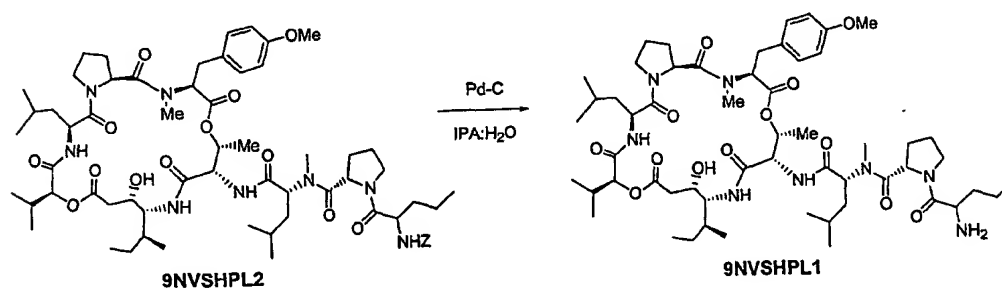
1 atm) with hydrogen gas while stirring for 14h. Then, the mixture was filtered (teflon 0.45  $\mu$ m) and concentrated under vacuum to yield the title compound (8.8 mg, quant) as a white solid.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.85-0.95 (m, 27H), 1.18-1.51 (m, 18H), 1.50-2.45 (m, 7H), 2.59-2.83 (m, 1H), 2.57 (s, 3H), 2.57-2.80 (m, 3H), 2.81-2.95 (m, 1H), 3.14 (s, 3H), 3.15-3.40 (m, 3H), 3.52-3.79 (m, 4H), 3.79 (s, 3H), 4.45-4.52 (m, 1 H), 4.61-4.65 (m, 1H), 4.70-4.85 (m, 2H), 5.17 (d,  $J = 3.3$ , 1H), 5.36-5.39 (m, 2H), 6.84 (d,  $J = 8.1$ , 2H), 7.86 (d,  $J = 8.7$ , 2H), 7.19 (d,  $J = 10.2$ , 1H), 7.82 (d,  $J = 9.0$ , 1H), 7.80-7.85 (m, 1H).

ESI-MS Calcd for  $\text{C}_{59}\text{H}_{94}\text{N}_8\text{O}_{14}$ : 1138.69. Found (m/z): 1139.7 [(M+H)] $^+$ .

#### Example 77

#### Synthesis of [Hiv] $^3$ -[NVa-Pro] $^9$ -aplidine (9NVSHPL1)



Following the procedure described for the synthesis of 9NVSAPL1, starting from 9NVSHPL2 (10 mg, 8.2  $\mu$ mol), the title compound (8 mg, quant) was obtained as a white solid.

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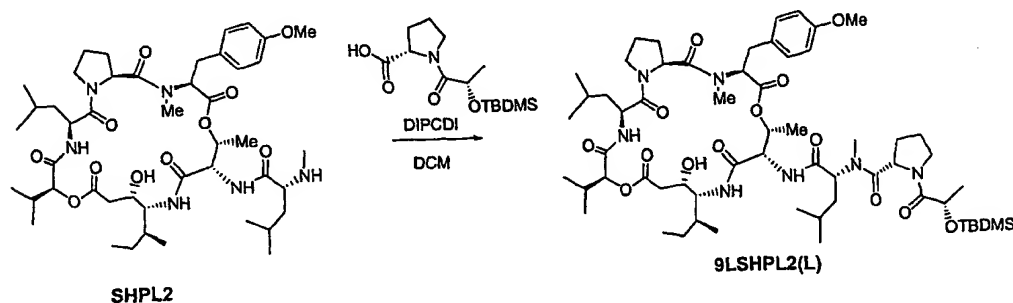
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-1.10 (m, 30H), 1.12-1.85 (m, 22H), 1.92-2.35 (m, 6H), 2.42 (m, 1H), 2.56 (s, 3H), 3.10-3.45 (m, 4H), 3.10 (s, 3H), 3.50-3.70 (m, 5H), 3.78 (s, 3H), 3.82 (m, 1H), 4.01 (m, 1H), 4.26 (m, 1H), 4.65 (m, 1H), 4.64 (m, 1H), 4.88 (m, 1H), 5.02 (d,  $J = 5.3$ , 1H), 5.32 (m, 2H), 6.84 (d,  $J = 8.3$ , 2H), 7.07 (d,  $J = 8.3$ , 2H), 7.38 (d,  $J = 8.7$ , 1H), 7.60 (d,  $J = 4.3$ , 1H), 7.80 (d,  $J = 9.3$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  12.03, 14.13, 14.30, 16.80, 17.81, 18.72, 19.15, 21.09, 21.55, 23.73, 23.96, 25.07, 26.16, 27.55, 28.17, 28.72, 29.91, 30.33, 31.52, 33.80, 34.17, 36.00, 38.94, 39.51, 39.84, 46.88, 47.51, 48.45, 55.01, 55.49, 56.91, 57.15, 58.10, 66.37, 69.17, 71.10, 79.12, 114.29, 130.21, 130.57, 158.82, 163.66, 168.91, 169.86, 170.38, 170.75, 170.82, 171.30, 173.22, 174.79.

ESI-MS Calcd for  $\text{C}_{56}\text{H}_{90}\text{N}_8\text{O}_{13}$  1082.66. Found  $m/z$ : 1083.7 ( $\text{M}+\text{H}$ ) $^+$ .

### Example 78

#### Synthesis of $[\text{Hiv}]^3\text{-[L-Lac(OTBDMS)]}^9\text{-aplidine [9LSHPL2(L)]}$ .



Following the procedure described for the synthesis of **SAPL1**, starting from **SHPL2** (10 mg, 11.2  $\mu\text{mol}$ ) and (L)-Lac(OTBDMS)-Pro-OH

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(17 mg, 56  $\mu$ mol), the title compound (9 mg, 68%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, gradient ACN/H<sub>2</sub>O 85:15-100:0 in 10 min. (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 30.1 min).

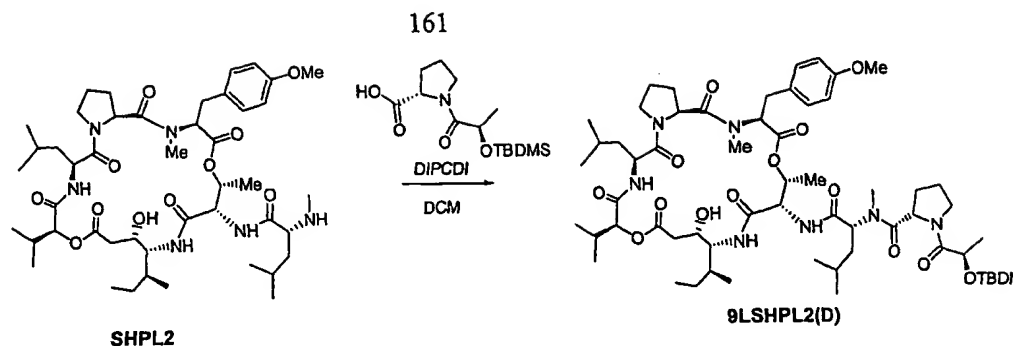
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.08 (s, 3H), 0.10 (s, 3H), 0.82-1.10 (m, 24H), 1.11-1.72 (m, 18H), 1.75-2.51 (m, 6H), 2.41 (m, 1H), 2.56 (s, 3H), 3.00-3.40 (m, 5H), 3.11 (s, 3H), 3.53-3.82 (m, 3H), 3.79 (s, 3H), 3.91 (m, 2H), 4.02 (m, 1H), 4.27 (m, 1H), 4.50 (m, 1H), 4.63 (m, 2H), 4.87 (m, 1H), 5.01 (d, J = 4.8, 1H), 5.27 (m, 2H), 6.84 (d, J = 8.7, 2H), 7.07 (d, J = 8.7, 2H), 7.29 (d, J = 9.7, 1H), 7.63 (d, J = 5.8, 1H), 7.88 (d, J = 9.7, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  -4.26, -4.12, 12.04, 14.40, 16.91, 17.97, 19.14, 20.60, 21.11, 21.66, 23.82, 24.11, 24.96, 25.08, 26.11, 26.37, 27.53, 28.18, 28.37, 30.33, 31.69, 33.75, 34.19, 36.23, 39.00, 39.36, 39.81, 46.87, 47.64, 48.45, 54.91, 55.37, 55.48, 56.81, 57.08, 58.37, 66.38, 69.14, 69.89, 71.42, 79.19, 82.66, 114.23, 130.36, 130.59, 158.77, 168.43, 469.86, 170.72, 171.01, 171.21, 172.03, 173.62, 174.91.

ESI-MS Calcd for C<sub>60</sub>H<sub>99</sub>N<sub>7</sub>O<sub>14</sub>Si: 1169.7. Found m/z: 1170.9 (M+H)<sup>+</sup>.

#### Example 79

**Synthesis of [Hiv]<sup>3</sup>-[D-Lac(OTBDMS)]<sup>9</sup>-aplidine [9LSHPL2(D)].**



Following the procedure described for the synthesis of **SAPL1**, starting from **SHPL2** (10 mg, 11.2  $\mu$ mol) and (D)-Lac(OTBDMS)-Pro-OH (17 mg, 56  $\mu$ mol), the title compound (9 mg, 68%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, gradient ACN/H<sub>2</sub>O 85:15-100:0 in 10 min (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 30.4 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.03 (m, 3H), 0.06 (m, 3H) 0.87 (s, 9H), 0.82-1.10 (m, 24H), 1.11-1.72 (m, 18H), 1.75-2.30 (m, 6H), 2.41 (m, 1H), 2.56 (s, 3H), 3.00-3.40 (m, 5H), 3.06 (s, 3H), 3.56 (m, 1H), 3.65 (m, 2H), 3.78 (s, 3H), 3.90 (m, 1H), 4.01 (m, 1H), 4.17 (m, 1H), 4.25 (m, 1H), 4.39 (m, 1H), 4.61 (m, 2H), 4.86 (m, 1H), 5.00 (d,  $J$  = 4.8, 1H), 5.25 (m, 2H), 6.84 (d,  $J$  = 8.3, 2H), 7.07 (d,  $J$  = 8.3, 2H), 7.29 (d,  $J$  = 9.7, 1H), 7.74 (d,  $J$  = 5.3, 1H), 7.87 (d,  $J$  = 9.7, 1H).

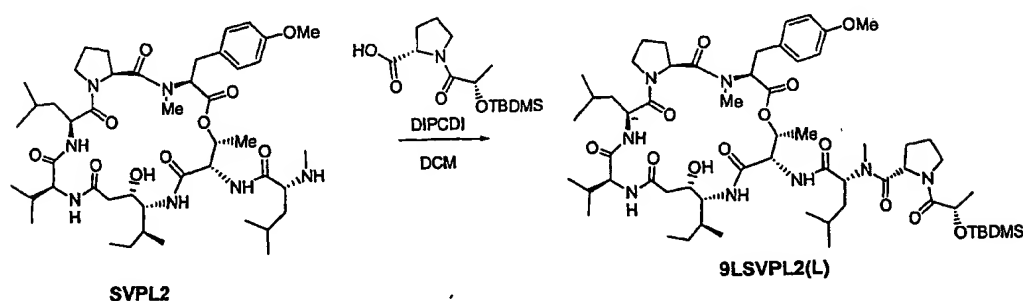
<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  -4.87, -4.84, 12.04, 14.38, 16.89, 17.94, 19.14, 20.24, 21.08, 21.60, 23.85, 24.12, 24.90, 25.06, 25.13, 26.01, 26.53, 27.53, 27.83, 28.20, 30.33, 31.51, 33.76, 34.20, 36.16, 38.99, 39.35, 39.82, 46.87, 47.02, 48.46, 54.79, 55.42, 55.48, 57.08, 57.18, 58.28, 66.39, 69.11, 71.43, 72.61, 79.18, 114.25, 130.29, 130.59, 158.79, 168.45, 169.86, 170.77, 170.81, 170.97, 171.20, 172.53, 173.67, 174.84.

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ESI-MS Calcd for  $C_{60}H_{99}N_7O_{14}Si$ : 1169.7. Found  $m/z$ : 1170.8  
( $M+H$ )<sup>+</sup>.

## Example 80

**Synthesis of [Val]<sup>3</sup>-[L-Lac(OTBDMS)]<sup>9</sup>-aplidine [9LSVPL2(L)].**



Following the procedure described for the synthesis of **SAPL1**, starting from **SVPL2** (10 mg, 11.2  $\mu$ mol) and (L)-Lac(OTBDMS)-Pro-OH (17 mg, 56  $\mu$ mol), the title compound (9 mg, 68%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 17.8 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  0.14 (s, 6H), 0.71-1.06 (m, 27H), 1.10-1.42 (m, 10H), 1.43-1.84 (m, 8H), 1.85-2.40 (m, 11H), 2.57 (s, 3H), 2.80 (d,  $J$  = 14.7, 1H), 3.15 (s, 3H), 3.15-3.23 (m, 1H), 3.33-3.42 (m, 2H), 3.54 (dd,  $J_1$  = 4.2,  $J_2$  = 10.8, 1H), 3.58-3.69 (m, 3H), 3.79 (s, 3H), 3.88-3.90 (m, 1H), 4.05-4.12 (bt, 1H), 4.32 (bs, 1H), 4.43-4.68 (m, 4H), 4.77 (t,  $J$  = 10.5, 1H), 5.31-5.35 (m, 2H), 6.83 (d,  $J$  = 8.4, 2H), 7.08 (d,  $J$  = 8.7, 2H), 7.39 (d,  $J$  = 9.9, 1H), 7.45 (d,  $J$  = 9.0, 1H), 7.60 (d,  $J$  = 10.5, 1H), 7.83 (d,  $J$  = 4.5, 1H).

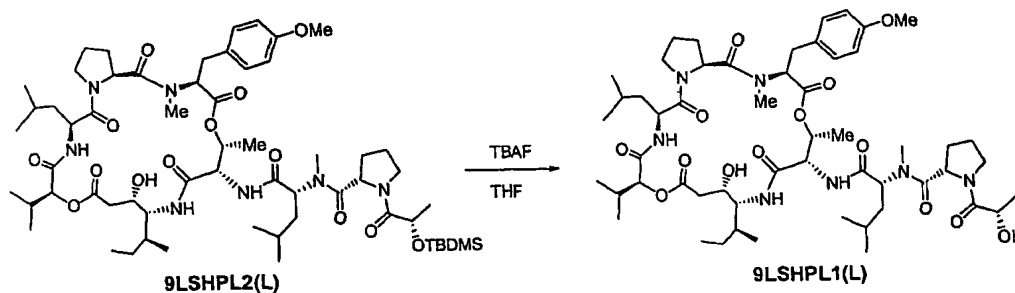
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$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  -4.67, -4.49, 11.84, 14.13, 16.67, 18.19, 18.41, 19.68, 20.48, 21.15, 21.40, 23.61, 23.92, 24.72, 24.80, 25.06, 25.88, 26.24, 27.17, 28.04, 28.13, 29.78, 31.57, 33.33, 33.81, 35.99, 38.84, 41.56, 41.95, 46.83, 47.52, 48.68, 54.09, 54.64, 55.26, 56.63, 57.13, 58.36, 59.12, 66.20, 70.10, 70.57, 71.27, 77.20, 114.01, 130.13, 130.36, 158.55, 168.09, 169.76, 170.07, 170.48, 170.77, 172.21, 172.33, 173.58, 175.45.

ESI-MS Cald. for  $\text{C}_{59}\text{H}_{40}\text{N}_8\text{O}_{14}$ : 1168.72. Found (m/z): 1169.8 (M+H) $^+$ .

#### Example 81

#### Synthesis of [Hiv] $^3$ -[L-Lac] $^9$ -aplidine [9LSHPL1(L)]: Tamandarine A.



To a solution of **9LSHPL2(L)** (16 mg, 14  $\mu\text{mol}$ ) in THF (500  $\mu\text{ml}$ , anhyd.) at 0°C under Ar, was added TBAF (50  $\mu\text{l}$ , 1M in THF). After 1 h at 22°C the mixture was concentrated in vacuo and the crude was purified by flash LC (silica gel, grad DCM:MeOH 1% to 5%) to yield the title compound 813 mg, 88%) as a white solid.

Experimental data were published: Fenical, W. *et al.*, *J. Org. Chem.* **2000**, 65, 782-792.

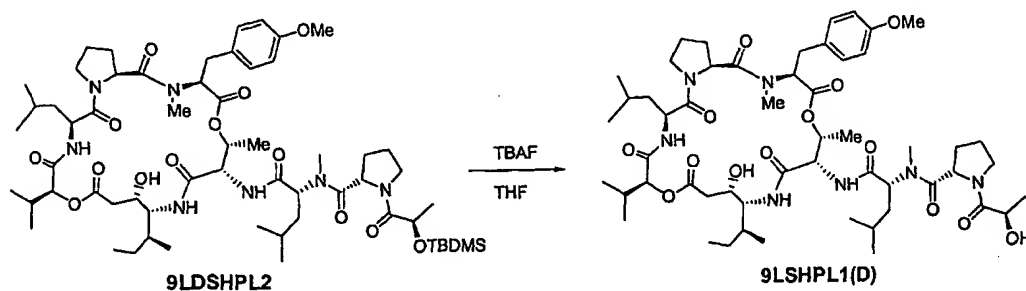


$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ ):  $\delta$  0.82-0.96 (m, 18H), 1.02 (d,  $J=3.4$ , 3H), 1.04 (d,  $J=3.4$ , 3H), 1.14-2.28 (m, 14H), 1.24 (s, 3H), 1.34 (d,  $J=6.8$ , 3H), 1.43 (d,  $J=6.8$ , 3H), 2.44 (dd,  $J_1=7.8$ ,  $J_2=17.1$ , 1H), 2.58 (s, 3H), 3.00 (bs, 1H), 3.10 (s, 3H), 3.14-3.31 (m, 2H), 3.37-3.43 (m, 2H), 3.56-3.72 (m, 5H), 3.79 (s, 3H), 3.90 (t,  $J=7.8$ , 1H), 4.02 (dt,  $J_1=3.4$ ,  $J_2=9.8$ , 1H), 4.25 (d,  $J=3.9$ , 1H), 4.30 (t,  $J=6.8$ , 1H), 4.37 (dd,  $J_1=7.3$ ,  $J_2=8.3$ , 1H), 4.65 (m, 1H), 4.71 (t,  $J=7.4$ , 1H), 4.87 (t,  $J=11.2$ , 1H), 5.03 (d,  $J=4.9$ , 1H), 5.29 (dd,  $J_1=3.4$ ,  $J_2=11.7$ , 1H), 5.42 (m, 1H), 6.83 (d,  $J=8.3$ , 2H), 7.07 (d,  $J=8.3$ , 2H), 7.34 (d,  $J=9.8$ , 1H), 7.48 (d,  $J=5.4$ , 1H), 7.76 (d,  $J=9.8$ , 1H).

ESI-MS Calcd for  $\text{C}_{54}\text{H}_{85}\text{N}_7\text{O}_{14}$ : 1055.6. Found: 1056.7 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 82

#### Synthesis of [Hiv] $^3$ -[D-Lac] $^9$ -aplidine [9LSHPL1(D)].



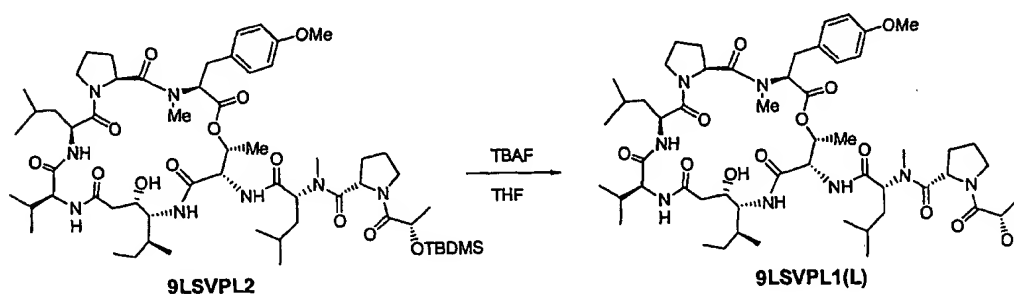
Following the procedure described for the synthesis of 9LSHPL1(L), starting from 9LSHPL2(D) (20 mg, 17  $\mu\text{mol}$ ) and TBAF (50  $\mu\text{l}$ , 1M in THF), afforded the title compound (14 mg, 78%) as a white solid, after purification by flash LC (silica gel, grad DCM:MeOH 1% to 5%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 0.78-1.08 (m, 18H), 1.02 (d, J=3.9, 3H), 1.04 (d, J=3.4, 3H), 1.10-2.36 (m, 14H), 1.20 (d, J=6.3, 3H), 1.34 (d, J=6.3, 3H), 1.37 (d, J=6.3, 3H), 2.38-2.50 (dd, J<sub>1</sub>=7.8, J<sub>2</sub>=17.5, 1H), 2.56 (s, 3H), 3.10 (s, 3H), 3.13-3.20 (m, 1H), 3.22-3.28 (m, 1H), 3.37-3.42 (dd, J<sub>1</sub>=3.9, J<sub>2</sub>=4.3, 1H), 4.61-4.68 (m, 3H), 3.69-3.76 (m, 3H), 3.77 (m, 1H), 3.78 (s, 3H), 3.90 (t, J=7.8, 1H), 3.97-4.07 (m, 1H), 4.26 (m, 1H), 4.41 (q, J=6.3, 1H), 4.63 (m, 1H), 4.71 (m, 1H), 4.86 (t, J=10.7, 1H), 5.01 (d, J=4.8, 1H), 5.21-5.37 (m, 2H), 6.83 (d, J=8.3, 2H), 7.06 (d, J=8.3, 2H), 7.41 (m, 2H), 7.77 (d, J=9.2, 1H).

ESI-MS Calcd for  $C_{54}H_{85}N_7O_{14}$ : 1055.62. Found: 1056.6 (M+H)<sup>+</sup>.

### Example 83

### Synthesis of [Val]<sup>3</sup>-[L-Lac]<sup>9</sup>-aplidine [9LSVPL1(L)].



Following the method described for the synthesis of **9LSHPL1**, starting from **9LSVPL2** (5 mg, 4.3  $\mu$ mol), afforded the little compound (4 mg, 88%) as a white solid, after purification by flash LC (silica gel, grad DCM:MeOH 1% to 5%).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>): δ 0.82-1.02 (m, 18H), 1.16-1.42 (m, 4H), 1.32 (d, J = 3.0, 3H), 1.40 (d, J = 6.6, 3H), 1.56-1.83 (m, 8H), 1.95-2.34

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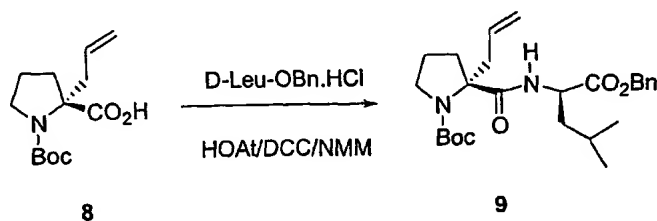
(m, 11H), 2.58 (s, 3H), 2.84 (d,  $J = 14.7$ , 1H), 3.15 (s, 3H), 3.15-3.23 (m, 1H), 3.36-3.42 (m, 1H), 3.55 (dd,  $J_1 = 9.0$ ,  $J_2 = 10.5$ , 2H), 3.64-3.66 (m, 3H), 3.95 (dd,  $J_1 = 3.3$ ,  $J_2 = 9.9$ , 1H), 4.08 (td,  $J_1 = 7.5$ ,  $J_2 = 17.1$ , 1H), 4.32 (bs, 1H), 4.41 (dd,  $J_1 = 6.6$ ,  $J_2 = 9.9$ , 1H), 4.48 (dd,  $J_1 = 5.1$ ,  $J_2 = 10.5$ , 1H), 4.61 (dd,  $J_1 = 6.0$ ,  $J_2 = 6.6$ , 1H), 4.69-4.80 (m, 2H), 5.29-5.35 (m, 1H), 5.57 (m, 1H), 6.84 (d,  $J = 8.1$ , 2H), 7.08 (d,  $J = 8.7$ , 2H), 7.37 (d,  $J = 3.9$ , 1H), 7.40 (d,  $J = 5.4$ , 1H), 7.60 (d,  $J = 10.8$ , 1H), 7.72 (d,  $J = 3.9$ , 1H).

ESI-MS Calcd for  $C_{54}H_{86}N_8O_{13}$ : 1054.63. Found: 1055.8 ( $M+H$ )<sup>+</sup>.

### Synthesis of the spiro[4,4]nonane unit:

#### Example 84

#### Synthesis of N-[(2R)-2-allyl-N-(tert-butoxycarbonyl)prolyl]D-leucine (9)



To a cooled (0°C) solution of **8** (1.53 g, 6 mmol) in anhyd DCM (33 ml) under argon, was added: HOAt (980 mg, 7.2 mmol), D-Leu-OBn.pTsOH (2.65 g, 12 mmol), NMM (1.21 g, 12 mmol) and DCC (1.48 g, 7.2 mmol). The mixture was stirred 2 h at 0°C and then 12 h at r.t.; additional D-Leu-OBn.pTsOH (0.66 g, 3 mmol) and NMM (0.30 g, 3 mmol) were added, and the mixture was stirred 3 h more. The mixture was filtered and the solvent was concentrated in vacuo. The residue was dissolved in EtOAc

(30 ml) and washed successively with  $\text{NaHCO}_3$  (2x25 ml, sat), citric acid (2x25 ml, 10%) and brine (25 ml). The organic solution was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated under reduced pressure. The residue was purified by LC-silica (hex-EtOAc, 6:1) to afford **9** (2.63 g, 96%) as a colourless oil.  $[\alpha]_{\text{D}}^{20}$  12.4° (c 1, MeOH). HPLC [column  $\square$  Bondapack C<sub>18</sub> (Waters), 10 m, 3.9x300 mm, flow: 1 ml/min, at 214 nm, eluent CAN/0.05% TFA (40:60)]  $t_{\text{R}}$  = 9.08 min].

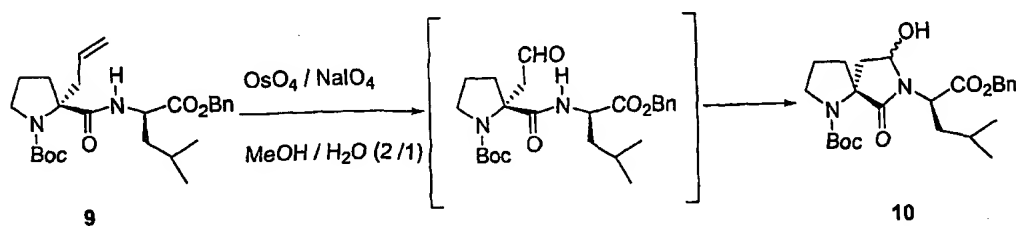
$^1\text{H-NMR}$  (300 MHz,  $\text{DMSO-d}_6$ )  $\delta$  0.86 (m, 6H), 1.37 (s, 9H), 1.55-1.72 (m, 5H), 2.00 (m, 2H), 2.64 (m, 1H), 2.86 (m, 1H), 3.15 (m, 1H), 3.55 (m, 1H), 4.41 (m, 1H), 5.05-5.11 (m, 4H), 5.63-5.72 (m, 1H), 7.28-7.35 (m, 5H).

$^{13}\text{C-MNR}$  (75 MHz, acetone- $\text{d}_6$ )  $\delta$  21.6, 22.8, 24.6, 28.3, 34.7, 38.2, 41.3, 49.38, 51.0, 66.9, 69.7, 80.1, 119.1, 128.3, 132.7, 153.9, 172.8.

Ref. Synthesis of **8**: a) Seebach, D. *et al. J. Am. Chem. Soc.* **1983**, 105, 5390-5398. b) Genin, M. J. *et al. J. Org. Chem.* **1993**, 58, 2334-2337.

#### Example 85

**Synthesis of (5R,8RS)-1-(tert-butoxycarbonyl)-7-[(1R)-1-benzyloxycarbonyl-3-methylbutyl]-8-hydroxy-6-oxo-1,7-diazaspiro[4.4]nonane (10)**



To a solution of **9** (1.56 g, 3.42 mmol) in MeOH/H<sub>2</sub>O (2:1, 108 ml) under argon, a solution of OsO<sub>4</sub> (2.5% w/w, 2.9 ml) in *tert*-butanol was added. Stirring was continued for 10 min and NaIO<sub>4</sub> (2.195 g, 10.3 mmol) was added. After 24 h of stirring the reaction mixture diluted with H<sub>2</sub>O (100 ml) and extracted with EtOAc (3x 50 ml). The combined organic extracts were washed with brine (50 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The residue was purified by LC-silica (hex-EtOAc, gradient 80:20 to 0:100%) to afford diastereomers **10a** and **10b** (combined: 1.17 g, 76%) as a white solid.

**10a**: HPLC [Column Novapack C<sub>18</sub> (Waters), 3.9 x 150 mm, □□=1ml / min, □=214nm, eluent: CH<sub>3</sub>CN / 0,05%TFA, (40/60)] t<sub>R</sub>=14.45 min. m.p.: 140-141°C. [α]<sub>D</sub><sup>20</sup> -4° (c 1, MeOH).

<sup>1</sup>H-NMR (300 MHz, acetone-d<sub>6</sub>) δ 0.90 (m, 6H), 1.29 (s, 9H), 1.64-2.30 (m, 9H); 2.68 (dd, 1H, J<sub>1</sub>= 6, J<sub>2</sub>=13, 1H), 3.37 (m, 2H), 4.51 (dd, 1H), 5.13 (d, J=15, 2H), 5.79 (t, J= 5, 2H), 7.40 (m, 5H).

<sup>13</sup>C-MNR (75 MHz, acetone-d<sub>6</sub>) δ 21.8, 23.8, 24.1, 24.9, 28.5, 39.6, 40.8, 41.5, 48.5, 66.8, 79.4, 79.8, 81.2, 129.1, 129.3, 128.6, 171.7, 172.0.

ESI-MS: Calcd for C<sub>25</sub>H<sub>36</sub>N<sub>2</sub>O<sub>6</sub>: 460.26. Found m/z: 483,4 (M+Na)<sup>+</sup>.

**10b**: HPLC [Column Novapack C<sub>18</sub> (Waters), 3.9x150 mm, □□=1ml / min, □=214nm, eluent: CH<sub>3</sub>CN / 0,05%TFA, (40/60)] t<sub>R</sub>=18.75 min. M.p.: 134-135°C. [α]<sub>D</sub><sup>20</sup> +26 (c 1.2, MeOH).

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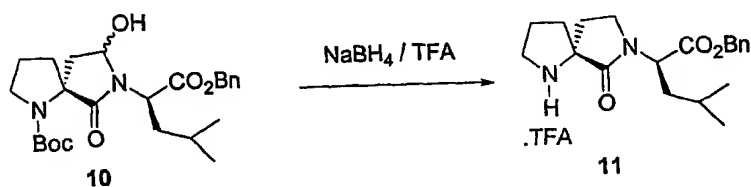
$^1\text{H-NMR}$  (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.90 (6H, m), 1.40 (9H, s), 1.50-2.60 (9H, m), 3.40 (2H, m), 4.20-5.40 (5H, m), 7.40 (5H, m).

$^{13}\text{C-MNR}$  (75 MHz, acetone- $d_6$ )  $\delta$  21.3, 23.2, 24.1, 24.9, 28.3, 38.9, 40.2, 42.5, 47.9, 53.2, 66.8, 77.5, 79.4, 80.6, 129.1, 171.2, 174.1.

ESI-MS Calcd for  $\text{C}_{25}\text{H}_{36}\text{N}_2\text{O}_6$ : 460.26. Found  $m/z$ : 483,5 ( $\text{M}+\text{Na}$ ) $^+$ .

## Example 86

**Synthesis of (5R)-7-[(1R)-1-benzoyloxycarbonyl-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane as trifluoroacetate salt (11)**



To a solution of **10** (430 mg, 0.93 mmol) in TFA (10 ml),  $\text{NaBH}_4$  (106 mg, 2.8 mmol) was added. The mixture was stirred for 2 h and then, the reaction was concentrated under reduced pressure. The residue was partitioned between  $\text{H}_2\text{O}$  (5 ml) and DCM (20 ml). The organic phase was dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated in vacuo to afford **11** as an orange oil (318 mg, quant.).  $[\alpha]_{20\text{D}} +15$  (c 1, MeOH).

$^1\text{H-NMR}$  (300 MHz, acetone- $d_6$ ) 0.91 (m, 6H), 1.50 (m, 1H), 1.66-1.94 (m, 2H), 2.11-2.72 (m, 6H), 3.48-3.72 (m, 4H), 4.74 (dd,  $J_1=6$ ,  $J_2=15$ , 1H), 5.18 (s, 2H), 7.37 (m, 5H).

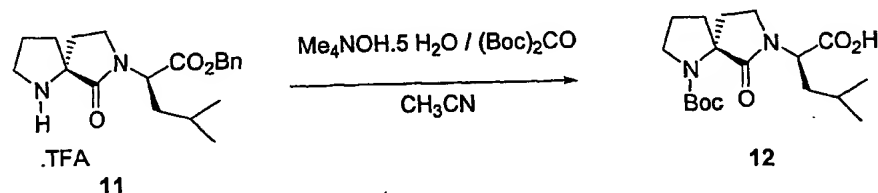
$^{13}\text{C-MNR}$  (75 MHz, acetone- $d_6$ )  $\delta$  21.2, 23.2, 23.9, 25.5, 30.2, 34.6,

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38.0, 42.2, 46.6, 53.9, 67.6, 69.6, 129.3, 161.1, 161.6, 170.9, 172.6.

ESI-MS Calcd for  $C_{20}H_{28}N_2O_3$ : 344.21. Found  $m/z$ : 345,3 ( $M+H$ )<sup>+</sup>.

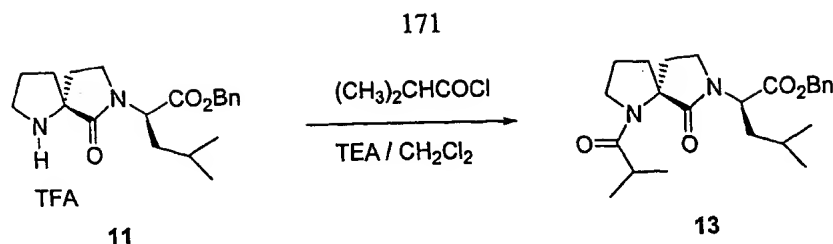
## Example 87

**Synthesis of (5R)-1-(tert-butoxycarbonyl)-7-[(1R)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane (12)**

To a solution of **11** (150 mg, 0.44 mmol) in ACN (5 ml), tetramethylammonium hydroxide pentahydrate (158 mg, 0.87 mmol) and  $Boc_2O$  (144 mg, 0.66 mmol) were added while stirring. After 6 h, additional TMAH. 5  $H_2O$  (158 mg) and  $Boc_2O$  (192 mg) were added. The reaction was stirred for 2d and then, it was partitioned between  $H_2O$  (10 ml) and DCM (25 ml). The aqueous phase was lyophilized and purified by LC-silica (DCM-MeOH, gradient 92:8 to 60:40) to yield **12** (100 mg, 64%) as a white solid.

## Example 88

**Synthesis of (5R)-1-(isobutyryl)-7-[(1R)-1-benzyloxycarbonyl-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane (13)**



To a solution of **11** (169 mg, 0.49 mmol) in anh DCM (10 ml) at 0° C under argon, were added TEA (199 mg, 1.96 mmol), DMAP (6 mg, 0.049 mmol) and dropwise isobutyryl chloride (104 mg, 0.98 mmol). The reaction mixture was allowed to warm to room temperature and stirred for 24 h. The crude was partitioned between H<sub>2</sub>O (10 ml) and DCM (10 ml). The organic phase was washed with brine (10 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated in vacuo. Pure compound **13** (150 mg, 74%) as a white solid, was obtained after LC-silica (hex-EtOAc, gradient 60:40 to 0:100).

HPLC [Column Novapack C<sub>18</sub> (Waters), 3.9 x 150 mm, □□=1ml /min, □=214 nm, eluyente: CH<sub>3</sub>CN / 0,05%TFA, (50/50)] t<sub>R</sub>= 4.50 min. M.p.: 87°C. [α]<sub>20D</sub> +9,6 (c 1.4, MeOH).

<sup>1</sup>H-NMR (300 MHz, CDCl<sub>3</sub>) δ 0.90 (2d, J= 7, 6H), 1.09-1.12 (2d, 6H), 1.37 (septuplet, J = 6, 1H), 1.61-2.10 (m, 7H), 2.64 (m, 2H), 3.14 (dd, J<sub>1</sub>=9, J<sub>2</sub>=17, 1H), 3.64 (m, 3H), 4.85 (dd, J<sub>1</sub> = 5, J<sub>2</sub> = 10, 1H), 5.14 (d, J = 6, 2H), 7.32 (m, 5H).

<sup>13</sup>C-NMR (300 MHz, CDCl<sub>3</sub>) δ 18.6, 18.7, □21.2, 23.1, 24.0, 24.8, 29.5, 32.5, 35.7, 37.6, 40.5, 47.8, 52.7, 66.7, 76.3, 170.8, 174.3, 174.9.

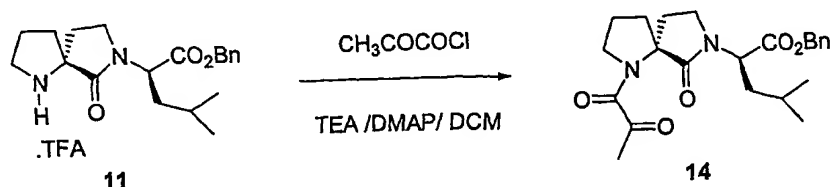
ESI-MS Calcd for C<sub>24</sub>H<sub>34</sub>N<sub>2</sub>O<sub>4</sub>: 414.25. Found m/z: 415,4 (M+H)<sup>+</sup>.

#### Example 89



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**Synthesis of (5R)-1-(pyruvyl)-7-[(1R)-1-benzyloxycarbonyl-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane (14)**



Pyruvil chloride was prepared according to the method described in the literature, Pansare, S.V.; Gnana R.R. " Asymmetric Allylation and reduction on an Ephedrine-Derived Template: Stereoselective Synthesis of  $\alpha$ -Hydroxy Acids and Derivatives" J. Org. Chem.1998, 63, 4120-4124.  $\alpha,\alpha$ -dichloromethyl methyl ether (188 mg, 1.57 mmol) was added to pyruvic acid (115 mg, 1.31 mmol). The reaction mixture was stirred for 20 min and the resulting solution was warmed to 50-55 °C and then, stirred for further 30 min. The reaction mixture was allowed to cool to room temperature and DCM (3 ml) was added.

To a solution of **11** (150 mg, 0.33 mmol) in anhyd DCM (4 ml) at 0° C under argon, were added TEA (200 mg, 1.98 mmol) and DMAP (4 mg, 0.033 mmol) to the freshly solution of pyruvil chloride at 0° C. The reaction mixture was allowed to warm to room temperature and stirred for 6 h. The crude was washed successively with citric acid (5 ml, 10%), aq. NaHCO<sub>3</sub> sat. (5ml) and brine (5 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated. Pure compound **14** (77 mg, 56%) as an oil, was obtained after LC-silica (hex-EtOAc, 1:3).

HPLC [Column Novapack C<sub>18</sub> (Waters), 3,9 x 150 mm,  $\square\square$ =1ml /min,  $\square$ =214nm, eluent: ACN / 0,05%TFA, (50/50)]  $t_R$ = 5,87 and 6.72 min.

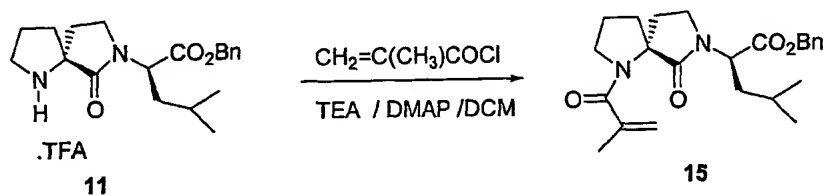
$^1\text{H}$ -MNR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  0.92 and 0.95 (2d,  $J = 6$ , 6H); 1.42 (m, 1H); 1.61-2.39 (m, 7H), 2.44 (s, 3H), 2.77 [m, 1H), 3.22 (m, 1H), 3.56-3.78 (m, 2H), 3.92 (m, 1H), 4.67 and 4.85 (dd,  $J_1 = 6$ ,  $J_2 = 10$ , 1H), 5.21 (s, 2H), 7.34 (m, 5H).

$^{13}\text{C}$ -MNR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  21.2, 23.0, 24.4, 24.8, 26.4, 29.3, 35.6, 37.3, 40.7, 48.9, 53.0, 66.8, 68.6, 135.0, 166.0, 170.8, 173.0, 198.0.

ESI-MS Calcd for  $\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_5$ : 414.22. Found  $m/z$ : 415.4 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 90

#### Synthesis of (5R)-1-(2-methylacryloyl)-7-[(1R)-1-benzyloxycarbonyl-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane (15)



Following the procedure described for the synthesis of **13**, starting from **11** (200 mg, 0.43 mmol) and methylacryloyl chloride (89 mg, 0.86 mmol), the title compound (70 mg, 50%) was obtained as a colourless oil, after purification by LC (silica ge, hex-EtOAc, 2:1). HPLC [Column Novapack C<sub>18</sub> (Waters), 3,9 x 150 mm,  $\square\square=1\text{ml /min}$ ,  $\square=214\text{nm}$ , eluent: ACN / 0,05%TFA, (25/75)]  $R_t=6.38$  min.

$^1\text{H}$ -MNR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  0.91 (t,  $J = 6$ , 6H), 1.44 (m, 1H), 1.64-1.93 (m, 5H), 1.93 (s, 3H), 1.96-2.12 (m, 2H), 2.78 (m, 1H), 3.20 (m,

174

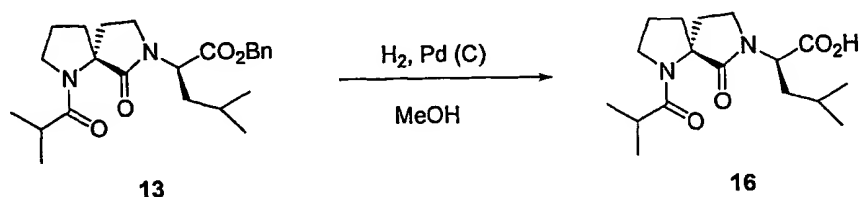
1H), 3.56-3.68 (m, 3H), 4.80 and 4.77 (2d,  $J = 10$ , 1H), 5.16 (s, 2H), 5.19 (d,  $J = 9$ , 2H), 7.32 (m, 5H).

$^{13}\text{C}$ -MNR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  19.8, 21.4, 23.0, 24.2, 24.9, 29.9, 37.1, 37.7, 41.1, 50.2, 53.2, 66.6, 67.4, 116.9, 128.1, 128.3, 128.4, 135.0, 141.7, 170.7, 174.1.

ESI-MS Calcd for  $\text{C}_{24}\text{H}_{32}\text{N}_2\text{O}_4$ : 412.24. Found: 413.3 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 91

#### Synthesis of (5R)-1-(isobutiryl)-7-[(1R)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane (**16**)



A degassed solution of **13** (134 mg, 0.32 mmol) containing 10% Pd/C (27 mg) was hydrogenated under 16 psi for 24 h. The mixture was filtered through a pad of celite and the filtered solution was concentrated under reduced pressure to afford **16** (100 mg, 95%) as a colourless oil. M.p. 68-69°C.  $[\alpha]_{\text{D}}^{20}$   $-2^\circ$  (c 1.1, MeOH).

$^1\text{H}$ -NMR [300 MHz, acetone- $\text{d}_6$ ]  $\delta$  0.87-0.91 (2d,  $J = 7$ , 6H), 1.09-1.12 (2d, 6H), 1.46 (m, 1H), 1.70 (m, 2H), 1.90-2.10 (m, 5H), 2.49 (m, 1H), 2.70 (m, 1H), 3.30 (m, 1H), 3.49 (dt,  $J_1 = 8$ ,  $J_2 = 10$ , 1H), 3.61-3.75 (m, 2H), 4.71 (dd,  $J_1 = 5$ ,  $J_2 = 11$ , 1H).

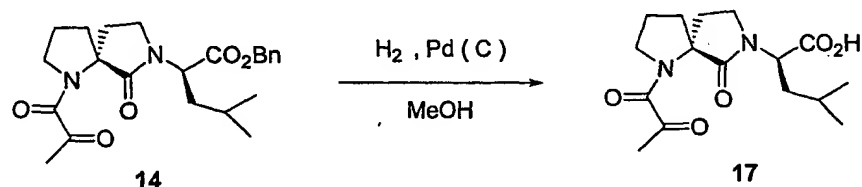
175

$^{13}\text{C}$ -NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  16.4, 16.6, 21.1, 23.0, 24.3, 25.0, 30.8, 32.6, 36.6, 36.9, 40.6, 48.0, 53.4, 67.5, 171.8, 174.2, 176.2.

ESI-MS Calcd for  $\text{C}_{17}\text{H}_{28}\text{N}_2\text{O}_4$ : 324.20. Found: 323.3 ( $\text{M}-1$ ) $^+$ .

### Example 92

#### Synthesis of (5*R*)-1-(pyruvil)-7-[(1*R*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane (17)



A degassed solution of **14** (79 mg, 0.26 mmol) in methanol (20 ml) containing Pd-C (10%, 22 mg) was hydrogenated under atmospheric pressure for 45 min. The filtered solution was concentrated under reduced pressure to afford **17** (79 mg, 95%) as a white solid. HPLC [Column Novapack  $\text{C}_{18}$  (Waters), 3.9 x 150 mm,  $\square\square=1\text{ml/min}$ ,  $\square=214\text{nm}$ , eluent: ACN / 0.05%TFA, (20/80)]  $\text{Rt}=13.14\text{ min}$ .

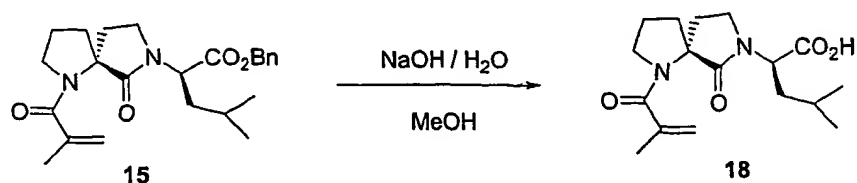
$^1\text{H}$ -MNR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  0.93 (m, 6H), 1.43 (m, 1H), 1.71-2.22 (m, 7H), 2.34 (s, 3H), 2.41 (s, 3H), 2.74 (m, 1H), 3.31 (m, 1H), 3.74 (m, 2H), 3.92 (m, 1H), 4.80 (dd,  $J_1=6$ ,  $J_2=10$ , 1H), 7.07 (bs, 1H).

$^{13}\text{C}$ -MNR (200 MHz,  $\text{CDCl}_3$ )  $\delta$  21.3, 23.2, 24.7, 25.1, 27.1, 29.9, 36.0, 37.0, 41.0, 49.3, 53.6, 69.1, 162.1, 172.7, 173.7, 197.5.

ESI-MS Calcd for  $\text{C}_{23}\text{H}_{30}\text{N}_2\text{O}_5$ : 324.17. Found  $m/z$ : 325.1

(M+H)<sup>+</sup>.

## Example 93

**Synthesis of (5R)-1-(2-methylacryloyl)-7-[(1R)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane (18)**

To a solution of **15** (65 mg, 0.16 mmol) in methanol (2.5 ml) were added aq. NaOH (1.6 ml, 1N) and H<sub>2</sub>O (1.6 ml) and the mixture was stirred at room temperature for 1 h. The resulting mixture was concentrated at reduced pressure, and the residue was partitioned between H<sub>2</sub>O (20 ml) and DCM (20 ml). The aqueous phase was acidified to pH=2 with aq HCl (10 ml, 0.1 N) and extracted with DCM (3x20 ml). The combined organic phases were washed with brine (25 ml), dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated at reduced pressure to afford **18** (44 mg, 85%) as a white solid. HPLC [Column Novapack C<sub>18</sub> (Waters), 3,9 x 150 mm, □□=1ml /min, □=214nm, eluent: ACN / 0,05%TFA, (25/75)] Rt= 6.38 min.

<sup>1</sup>H-MNR (300 MHz, CDCl<sub>3</sub>) δ 0.86 (d, J = 5, 3H), 0.89 (d, J = 5, 3H), 1.42 (m, 1H), 1.55-2.25 (m, 8H), 2.56 (m, 1H), 3.19-3.44 (m, 2H), 3.57-3.67 (m, 2H), 4.78 (d, J = 11, 1H), 4,82 (d, J = 11, 1H), 5.18 (d, J = 6, 2H).

<sup>13</sup>C-MNR (75 MHz, CDCl<sub>3</sub>) δ 19.5, 21.2, 23.1, 24.2, 25.1, 30.9,

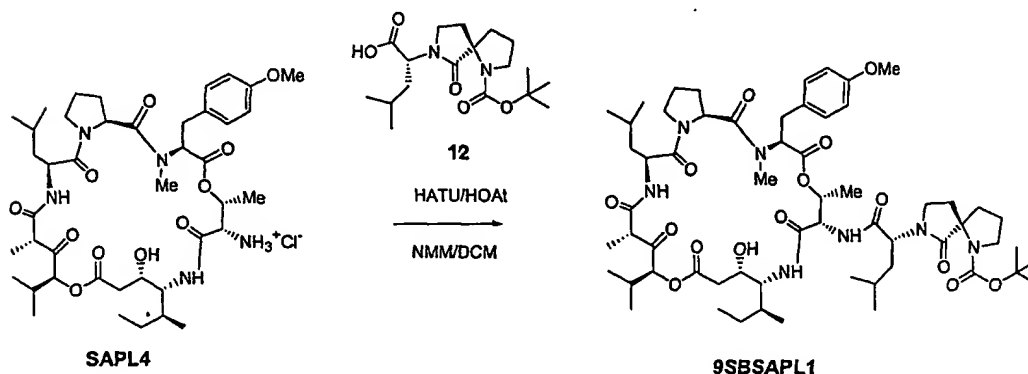
177

36.5, 37.6, 40.9, 50.5, 53.7, 67.5, 117.5, 140.6, 170.8, 174.2.

ESI-MS Calcd for  $C_{17}H_{26}N_2O_4$ : 322.19. Found: 323.2 (M+H)<sup>+</sup>.

## Example 94

**Synthesis of [(5*R*)-1-(tert-butoxycarbonyl)-7-[(1*R*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane]<sup>7-9</sup>-aplidine (9SBSAPL1).**



Following the procedure described for the synthesis of **SAPL3**, starting from **SAPL4** (10 mg, 13  $\mu$ mol), **12** (5 mg, 14  $\mu$ mol), HATU (12.4 mg), HOAt (4.5 mg), NMM (3.3  $\mu$ l), DCM (140  $\mu$ l) and DMF (70  $\mu$ l), the title compound (11 mg, 73%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm, Rt = 30 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  0.85-0.97 (m, 24 H), 1.19-1.34 (m, 18 H), 1.48 (s, 9H), 1.50-2.20 (m, 6 H), 2.32-2.36 (m, 1H), 2.54 (s, 3H), 2.58-2.72 (m, 2H), 2.97-3.08 (m, 1H), 3.10-3.22 (m, 3H), 3.34 (dd, 1H, J = 3.9, 13.8), 3.46-3.79 (m, 6H), 3.79 (s, 3 H), 4.03-4.12 (m, 2H), 4.28 (dd, 1H, J

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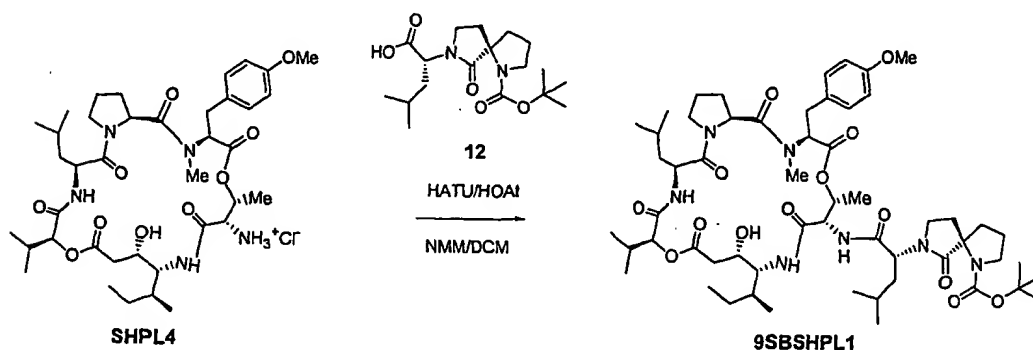
= 6.6, 13.2), 4.57-4.63 (m, 2H), 4.79-4.88 (m, 2H), 5.15 (d, 1H,  $J = 3.3$ ), 5.21-5.23 (m, 1H), 6.84 (d, 2 H,  $J = 8.4$ ), 7.07 (d, 2H,  $J = 8.7$ ), 7.28 (d, 1H,  $J = 10.8$ ), 7.79 (d, 1H,  $J = 6.6$ ), 7.82 (d, 1H,  $J = 9.9$ ).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.66, 15.21, 15.43, 16.79, 17.23, 18.69, 18.77, 21.24, 23.67, 23.95, 24.10, 24.90, 25.10, 25.38, 27.04, 28.23, 28.75, 29.93, 31.61, 34.50, 33.88, 34.16, 36.20, 36.57, 38.76, 39.09, 39.78, 41.29, 47.27, 47.76, 49.60, 49.96, 52.66, 55.50, 56.26, 57.38, 58.18, 66.74, 66.86, 68.34, 70.53, 80.75, 81.93, 114.33, 130.23, 130.54, 168.08, 169.83, , 170.18, 170.80, 171.43, 172.55, 175.04, 205.04.

ESI-MS Calcd for  $\text{C}_{60}\text{H}_{93}\text{N}_7\text{O}_{15}$  1151.7. Found  $m/z$ : 1152.4 ( $\text{M}+\text{H}$ ) $^+$

#### Example 95

**Synthesis of [Hiv] $^3$ -[(5*R*)-1-(tert-butoxycarbonyl)-7-[(1*R*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane] $^{7-9}$ -aplidine (9SBSHPL1).**



Following the procedure described for the synthesis of **SAPL3**, starting from **SHPL4** (10 mg, 13  $\mu\text{mol}$ ), **12** (5 mg, 14  $\mu\text{mol}$ ), HATU (14

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mg), HOAt (5 mg), NMM (6  $\mu$ l), DCM (150  $\mu$ l) and DMF (50  $\mu$ l), the title compound (10 mg, 70%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm, Rt = 28.1 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.80-1.07 (m, 24H), 1.08-1.67 (m, 12H), 1.48 (s, 9H), 1.68-2.30 (m, 10H), 2.41 (m, 1H), 2.55 (s, 3H), 2.68 (m, 1H), 2.94 (m, 1H), 3.07-3.40 (m, 4H), 3.42-3.72 (m, 6H), 3.78 (s, 3H), 3.90 (m, 1H), 4.01 (m, 1H), 4.29 (m, 1H) 4.63 (m, 1H), 4.77 (m, 1H), 4.87 (m, 1H), 5.02 (d, J= 4.8, 1H), 5.25 (m, 1H), 6.84 (d, J= 8.3, 2H), 7.07 (d, J= 8.3, 2H), 7.31 (d, J= 9.7, 1H), 7.50 (d, J= 5.8, 1H), 7.85 (d, J= 9.7, 1H).

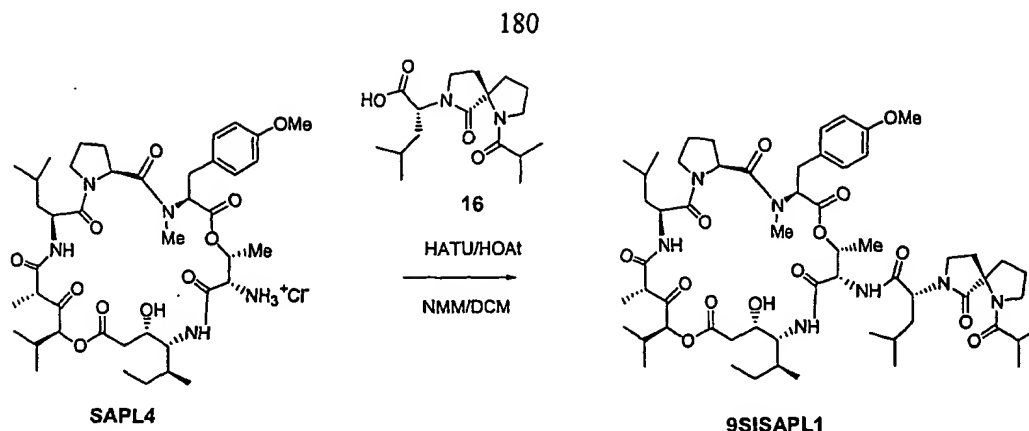
<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  12.07, 14.25, 17.12, 17.92, 19.15, 21.11, 21.18, 23.80, 24.00, 24.06, 24.86, 25.08, 25.15, 27.58, 28.21, 28.82, 30.33, 31.18, 33.78, 34.28, 35.77, 36.65, 39.07, 39.45, 39.86, 46.91, 48.14, 48.47, 52.62, 55.49, 57.13, 58.42, 66.36, 66.80, 69.14, 70.84, 79.19, 80.55, 114.27, 130.29, 130.59, 154.12, 158.81, 168.25, 169.84, 170.72, 170.80, 170.90, 171.25, 174.89.

ESI-MS Calcd for C<sub>57</sub>H<sub>89</sub>N<sub>7</sub>O<sub>14</sub> 1095.6. Found m/z: 1096.9 (M+H)<sup>+</sup>.

#### Example 96

**Synthesis of [(5R)-1-(isobutiryl)-7-[(1R)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane]<sup>7-9</sup>-Aplidine (9SISAPL1).**





Following the procedure described for the synthesis of **SAPL3**, starting from **SAPL4** (11 mg, 12.9  $\mu$ mol), **16** (5 mg, 15.4  $\mu$ mol), HATU (14 mg), HOAt (5 mg), NMM (3.6  $\mu$ l), DCM (155  $\mu$ l) and DMF (78  $\mu$ l), the title compound (10 mg, 69%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 19 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  0.86-1.00 (m, 24H), 1.12 (d,  $J$  = 6.9, 3H), 1.18 (d,  $J$  = 6.6, 3H), 1.34 (t,  $J$  = 6.6, 2H), 0.90-1.30 (m, 7H), 1.56-2.25 (m, 16 H), 2.30-2.80 (m, 3H), 2.55 (s, 3H), 2.95-3.06 (m, 1H), 3.15-3.25 (m, 3 H), 3.65 (dd, 1H) 3.52-3.79 (m, 6H), 3.79 (s, 3H), 3.98-4.15 (m, 1H), 4.28 (dd,  $J_1$  = 6.6,  $J_2$ =10.3, 1H), 4.59 (m, 2H), 4.79-4.85 (m, 2H), 5.17 (d,  $J$  = 3.6, 1H), 5.40-5.44 (m, 1H), 6.84 (d,  $J$  = 8.4, 2H), 7.06 (d,  $J$  = 8.7, 2H), 7.24 (d,  $J$  = 11.1, 1H), 7.90 (d,  $J$  = 9.3, 1H), 8.56 (d,  $J$  = 5.1, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$ : 11.50, 14.92, 15.22, 16.68, 16.95, 18.50, 18.59, 18.81, 20.94, 23.42, 23.80, 24.49, 24.68, 24.90, 25.11, 26.91, 27.98, 30.93, 31.30, 35.58, 33.88, 34.16, 35.85, 36.24, 38.64, 38.84, 39.71, 41.29, 47.01, 47.76, 49.42, 49.62, 52.66, 55.26, 55.73, 57.12, 58.21, 66.57, 67.40, 68.10, 70.79, 81.57, 114.07, 130.05, 130.31,

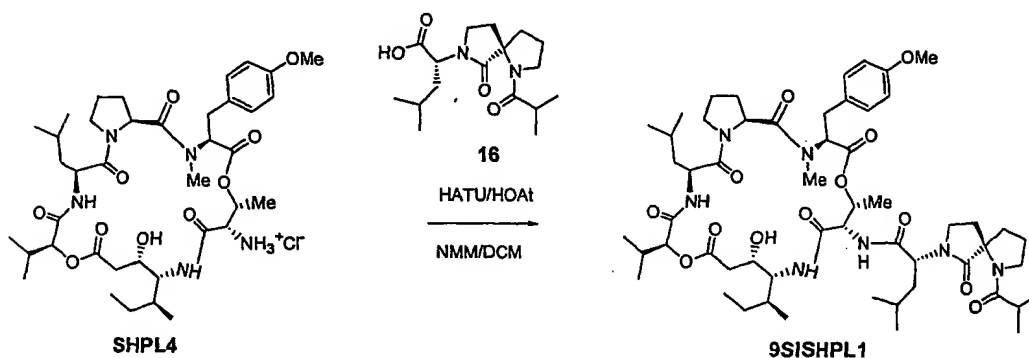
181

158.57, 168.12, 169.66, 170.08, 170.56, 171.13, 171.96, 172.37, 174.04, 175.41, 205.04.

ESI-MS Calcd for  $C_{59}H_{91}N_7O_{14}$ : 1121.66. Found: 1122.8 (M+H)<sup>+</sup>.

### Example 97

**Synthesis of [Hiv]<sup>3</sup>-[(5*R*)-1-(tert-butoxycarbonyl)-7-[(1*R*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane]<sup>7-9</sup>-aplidine (9SISHPL1).**



Following the procedure described for the synthesis of **SAPL3**, starting from **SHPL4** (10 mg, 13  $\mu$ mol), **16** (4.5 mg, 14  $\mu$ mol), HATU (14 mg), HOAt (5 mg), NMM (6  $\mu$ l), DCM (150  $\mu$ l) and DMF (50  $\mu$ l), the title compound (10 mg, 72%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 16.9 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.80-1.07 (m, 24H), 1.08-1.47 (m, 12H), 1.48-2.30 (m, 16H), 2.36 (m, 2H), 2.56 (s, 3H), 2.65 (m, 1H), 2.96 (m, 1H), 3.18 (m, 2H), 3.36 (m, 2H), 3.65 (m, 6H), 3.78 (s, 3H), 3.91 (m, 1H), 4.02 (m, 1H), 4.25 (m, 1H), 4.63 (m, 1H), 4.72 (m, 2H), 4.87 (m, 1H),

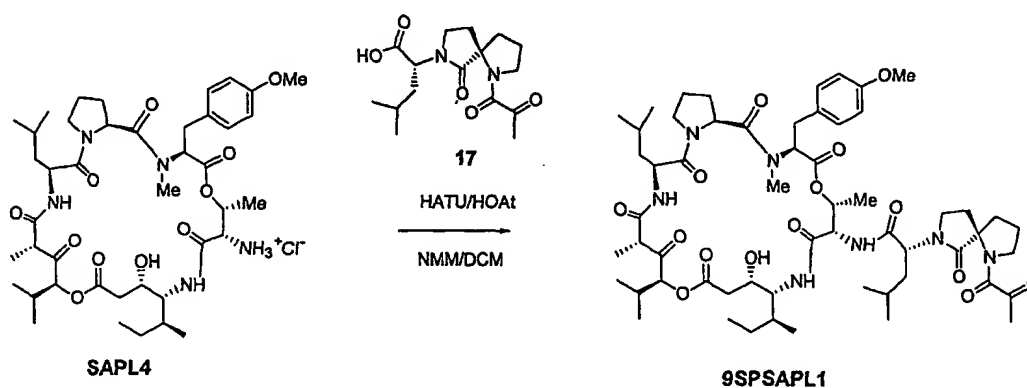
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5.02 (d, J= 4.8, 1H), 5.45 (m, 1H), 6.84 (d, J= 8.7, 2H), 7.07 (d, J= 8.7, 2H), 7.27 (d, J= 4.8, 1H), 7.88 (d, J= 9.7, 1H), 8.32 (d, J= 4.8, 1H).

ESI-MS Calcd for C<sub>56</sub>H<sub>87</sub>N<sub>7</sub>O<sub>13</sub> 1065.6. Found m/z: 1066.7 (M+H)<sup>+</sup>.

### Example 98

#### Synthesis of [(5R)-1-(pyruvyl)-7-[(1R)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane]<sup>7-9</sup>-aplidine (9SPSAPL1).



Following the procedure described for the synthesis of **SAPL3**, starting from **SAPL4** (10 mg, 11.7  $\mu$ mol), **17** (5 mg, 15.4  $\mu$ mol), HATU (12 mg), HOAt (5 mg), NMM (5  $\mu$ l), DCM (140  $\mu$ l) and DMF (70  $\mu$ l), the title compound (9 mg, 63%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm, t<sub>R</sub> = 14.4 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>):  $\delta$  0.90-1.00 (m, 24 H), 1.05-1.40 (m, 12 H), 1.40-2.25 (m, 16H), 2.27-2.41 (m, 1H), 2.42-2.70 (m, 3H), 2.54 (s, 3H), 2.92-2.98 (m, 1H), 3.12-3.38 (m, 4 H), 3.54-3.78 (m, 4 H), 3.79 (s,

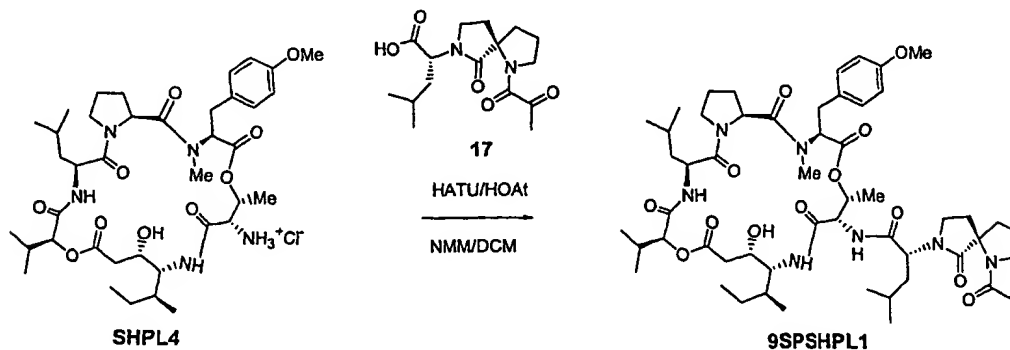
3H), 4.01-4.12 (m, 2H), 4.20-4.26 (m, 2H), 4.57-4.62 (m, 2H), 4.77-4.82 (m, 2H), 5.35.18 (d,  $J = 3.0$ , 2H), 5.37-5.42 (m, 1H), 6.84 (d,  $J = 8.7$ , 2H), 7.07 (d,  $J = 8.7$ , 2H), 7.20 (d,  $J = 9.6$ , 1H), 7.85 (d,  $J = 9.6$ , 1H), 98.04 (d,  $J = 5.4$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.86, 14.97, 15.47, 16.78, 17.12, 18.84, 21.20, 21.27, 23.62, 24.14, 15.03, 25.15, 25.30, 27.35, 27.51, 28.19, 30.46, 31.52, 34.27, 35.95, 39.01, 40.07, 41.59, 47.25, 49.72, 53.08, 55.51, 55.81, 57.38, 58.21, 66.66, 68.19, 69.23, 70.74, 81.69, 85.15, 114.33, 130.13, 130.56, 158.85, 161.12, 168.49, 169.81, 169.91, 170.81, 171.38, 171.69, 172.60, 173.34, 197.64, 205.19.

ESI-MS Calcd for  $\text{C}_{58}\text{H}_{87}\text{N}_7\text{O}_{15}$ : 1121.63. Found 1122.3 (M+H) $^+$ .

#### Example 99

**Synthesis of [Hiv] $^3$ -[(5R)-1-(pyruvyl)-7-[(1R)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane] $^{7-9}$ -aplidine (9SPSHPL1).**



Following the procedure described for the synthesis of **SAPL3**, starting from **SHPL4** (10 mg, 13  $\mu\text{mol}$ ), **17** (4.5 mg, 14  $\mu\text{mol}$ ), HATU (14 mg), HOAt (5 mg), NMM (6  $\mu\text{l}$ ), DCM (150  $\mu\text{l}$ ) and DMF (50  $\mu\text{l}$ ), the title compound (10 mg, 72%) was obtained as a white solid after purification

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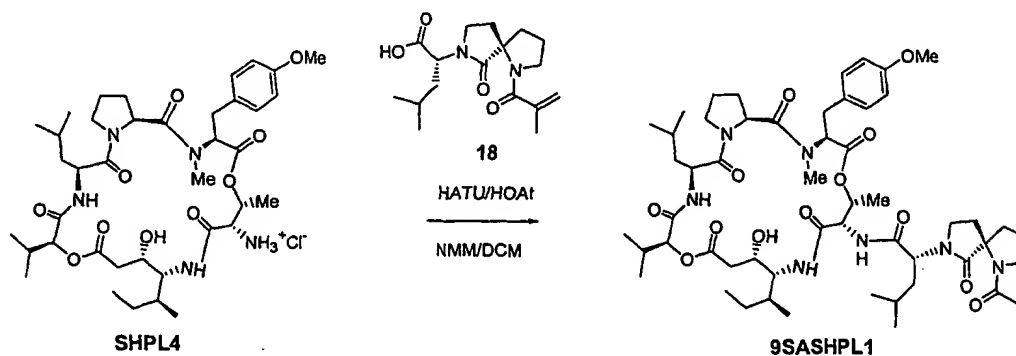
by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 13.6 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.80-1.10 (24H, m), 1.11-1.80 (12H, m), 1.81-2.30 (10H, m), 2.45 (m, 1H), 2.55 (s, 3H), 2.57 (s, 3H), 3.07-3.43 (m, 6H), 3.52-3.77 (m, 6H), 3.78 (s, 3H), 3.91 (m, 1H), 4.03 (m, 1H), 4.29 (m, 1H), 4.63 (m, 1H), 4.72 (m, 1H), 4.87 (m, 1H), 5.03 (d,  $J$  = 4.3, 1H), 5.45 (m, 1H), 6.84 (d,  $J$  = 8.3, 2H), 7.07 (d,  $J$  = 8.3, 2H), 7.29 (d,  $J$  = 8.7, 1H), 7.81 (d,  $J_1$  = 9.2, 1H), 7.87 (d,  $J$  = 4.8, 1H).

ESI-MS Calcd for C<sub>55</sub>H<sub>83</sub>N<sub>7</sub>O<sub>14</sub>: 1065.6. Found 1066.4 (M+H)<sup>+</sup>.

#### Example 100

**Synthesis of [Hiv]<sup>3</sup>-[(5*R*)-1-(acriloyl)-7-[(1*R*)-1-carboxy-3-methylbutyl]-6-oxo-1,7-diazaspiro[4.4]nonane]<sup>7-9</sup>-aplidine (9SASHPL1).**



Following the procedure described for the synthesis of **SAPL3**, starting from **SHPL4** (10 mg, 13  $\mu$ mol), **18** (5.8 mg, 14  $\mu$ mol), HATU (14 mg), HOAt (5 mg), NMM (6  $\mu$ l), DCM (150  $\mu$ l) and DMF (50  $\mu$ l), the title compound (10 mg, 72%) was obtained as a white solid after purification

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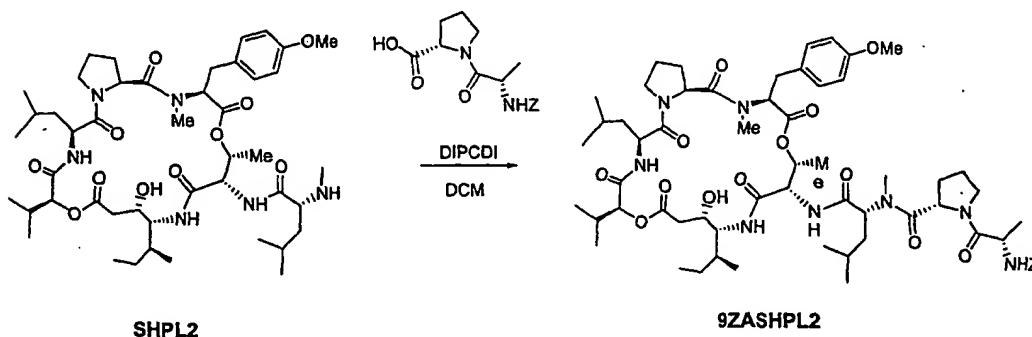
by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 16.4 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.85-0.96 (m, 18H), 1.02-1.05 (m, 6H), 1.14-1.45 (m, 12H), 1.49-1.64 (m, 4H), 1.68-1.77 (m, 1H), 1.89-2.05 (m, 3H), 1.99 (s, 3H), 2.10-2.28 (m, 4H), 2.43 (dd,  $J_1=7.8$ ,  $J_2=17.1$ , 1H), 2.57 (s, 3H), 2.60-2.68 (m, 1H), 2.97 (bs, 1H), 3.13-3.40 (m, 4H), 3.54-3.77 (m, 5H), 3.79 (s, 3H), 3.89-4.07 (m, 2H), 4.27 (m, 1H), 4.64 (m, 1H), 4.73 (m, 1H), 4.88 (m, 1H), 5.03 (d,  $J=4.4$ , 1H), 5.30 (d,  $J=20$ , 1H), 5.30-5.39 (m, 1H), 6.84 (d,  $J=8.3$ , 2H), 7.08 (d,  $J=8.3$ , 2H), 7.29 (s, 1H), 7.88 (d,  $J=9.8$ , 1H), 8.23 (d,  $J=7.4$ , 1H).

ESI-MS Calcd for C<sub>56</sub>H<sub>85</sub>N<sub>7</sub>O<sub>13</sub>: 1063.6. Found 1064.6 (M+H)<sup>+</sup>.

#### Example 101

#### Synthesis of [Hiv]<sup>3</sup>-[Z-Ala]<sup>9</sup>-aplidine (9ZASHPL2).



To a flask containing Z-Ala-Pro-OH (36 mg, 112  $\mu$ mol) in DCM (0.4 ml) at 0° C, under argon, DIPCPI (10  $\mu$ l, 64  $\mu$ mol) was added and the mixture was stirred for 60 min. Then, a solution of **SHPL2** (20 mg, 22.5  $\mu$ mol) in DCM (0.2 ml) was added and after 3d the reaction was quenched by addition of aq HCl (3 ml, 0.1 N). The mixture was stirred

for 5 min and then, diluted with DCM (4 ml) and washed successively with aq. KHSO<sub>4</sub> (4 ml, 10%), aq. NaHCO<sub>3</sub> (4 ml, sat) and brine (4 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. Purification of the residue by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm, t<sub>R</sub> = 18.2 min) afforded **9ZASHPL2** (30 mg, 67%) as a white solid.

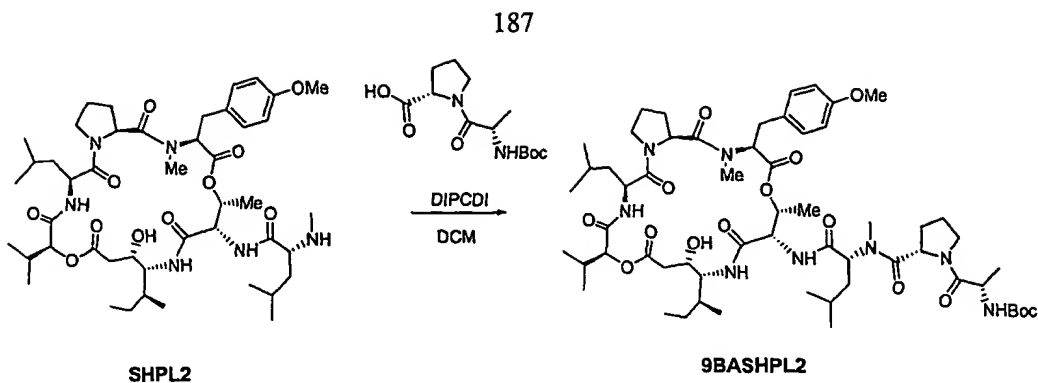
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.82-1.10 (m, 24H), 1.12-1.50 (m, 18H), 1.52-2.70 (m, 6H), 2.45 (m, 1H), 2.56 (s, 3H), 2.96-3.38 (m, 4H), 3.10 (s, 3H), 3.52-3.72 (m, 5H), 3.85 (m, 1H), 3.78 (s, 3H), 4.01 (m, 1H), 4.18 (m, 1H), 4.51 (m, 1H), 4.64 (m, 1H), 4.71 (m, 1H), 4.87 (m, 1H), 5.02 (d, J<sub>1</sub> = 5.3, 1H), 5.06 (m, 2H), 5.25 (m, 1H), 5.42 (m, 1H), 6.10 (d, J = 8.3, 1H), 6.84 (d, J = 8.3, 2H), 7.07 (d, J = 8.3, 2H), 7.31 (m, 6H), 7.70 (d, J = 4.3, 1H), 7.77 (d, J = 9.7, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>) δ 11.80, 13.97, 16.63, 17.02, 17.48, 18.93, 20.86, 21.15, 23.45, 23.76, 24.78, 25.96, 27.31, 27.92, 28.50, 30.06, 31.36, 33.44, 33.82, 35.66, 38.70, 39.38, 39.63, 46.54, 46.62, 46.98, 47.10, 48.19, 48.59, 54.89, 54.97, 55.22, 56.44, 56.90, 58.14, 66.16, 66.36, 68.95, 70.94, 78.78, 114.00, 127.75, 128.30, 129.90, 130.26, 156.31, 158.53, 168.67, 169.57, 170.06, 170.25, 170.65, 171.97, 173.08, 174.67.

ESI-MS Calcd for C<sub>62</sub>H<sub>92</sub>N<sub>8</sub>O<sub>15</sub> 1188.67. Found m/z 1189.7 (M+H)<sup>+</sup>.

#### Example 102

#### Synthesis of [Hiv]<sup>3</sup>-[Boc-Ala]<sup>9</sup>-aplidine (**9BASHPL2**).



Following the procedure described for the synthesis of **9ZASHPL2**, from **SHPL2** (10 mg, 11.2  $\mu$ mol), Boc-Ala-Pro-OH (17 mg, 57  $\mu$ mol), DIPCPI (5  $\mu$ l) and DCM (300  $\mu$ l), the title compound (9 mg, 70%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, gradient ACN/H<sub>2</sub>O 85:15-100:0 in 10 min (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 14.5 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.82-1.10 (m, 24H), 1.12-1.50 (m, 15H), 1.40 (s, 9H), 1.52-2.70 (m, 9H), 2.45 (m, 1H), 2.60 (s, 3H), 3.00-3.43 (m, 4H), 3.10 (s, 3H), 3.62 (m, 5H), 3.79 (s, 3H), 3.90 (m, 1H), 4.02 (m, 1H), 4.20 (m, 1H), 4.41 (m, 1H), 4.67 (m, 2H), 4.87 (m, 1H), 5.02 (d,  $J_1$  = 4.3, 1H), 5.26 (m, 1H), 5.40 (m, 1H), 5.76 (d,  $J$  = 7.8, 1H), 6.84 (d,  $J$  = 8.3, 2H), 7.07 (d,  $J$  = 8.3, 2H), 7.32 (d,  $J$  = 9.7, 1H), 7.73 (d,  $J$  = 4.8, 1H), 7.80 (d,  $J$  = 9.7, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  12.06, 14.34, 16.88, 17.20, 17.77, 19.17, 21.10, 21.41, 23.72, 24.03, 25.06, 26.17, 27.60, 28.19, 28.60, 30.31, 31.63, 33.74, 35.96, 38.96, 38.93, 39.59, 39.86, 46.86, 47.27, 48.29, 48.45, 55.18, 55.42, 55.49, 56.59, 57.16, 58.44, 66.43, 69.14, 71.30, 79.05, 79.40, 114.29, 130.21, 130.54, 156.06, 158.83, 168.80, 169.87, 170.51, 170.64, 170.94, 171.31, 172.59, 173.45, 174.92.

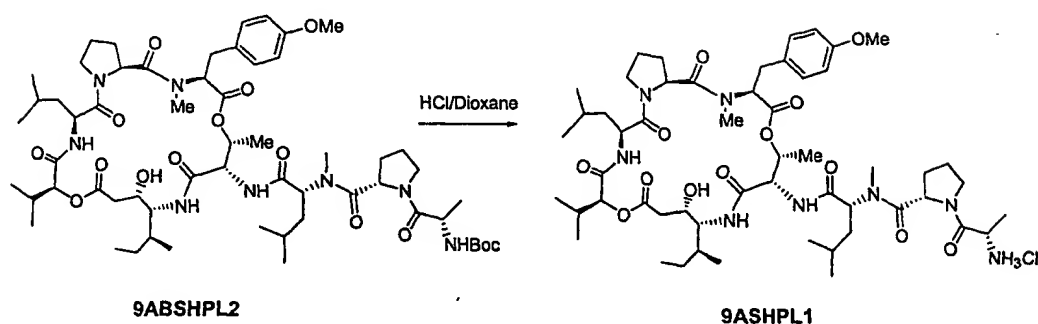


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ESI-MS Calcd for  $C_{59}H_{94}N_8O_{15}$  1154.68. Found  $m/z$  1155.6  
(M+H)<sup>+</sup>.

### Example 103

### Synthesis of [Hiv]<sup>3</sup>-[Ala]<sup>9</sup> aplidine (9ASHPL1).



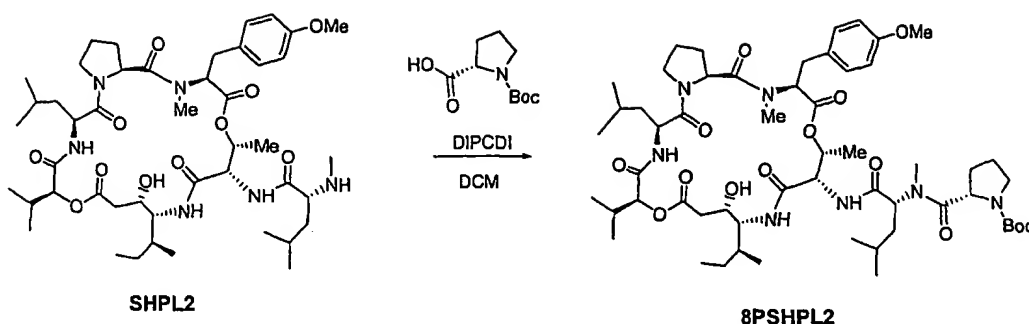
To a flask containing **9BASHPL2** (8 mg, 6.92  $\mu$ mol), a solution of hydrochloric acid in anhydrous dioxane (1.5 ml, 5.3 N, 7.9 mmol) was added. The resulting solution was stirred at room temperature for 5 h or until complete disappearance of the starting material (TLC). Then, the solution was concentrated under reduced pressure and the residue was dissolved in DCM and concentrated again. The white foam crude was precipitated with DCM /hex (2 ml/4 ml) to yield **9ASHPL1** (7.2 mg, quant.) as a white solid.

ESI-MS Calcd for  $C_{54}H_{86}N_8O_{13}$  1054.6. Found  $m/z$ : 1055.6  
( $M+H$ )<sup>+</sup>.

### Example 104

### Synthesis of [Hiv]<sup>3</sup>-[Boc-Pro]<sup>8</sup>-didemnin A (8PSHPL2)

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Following the procedure described for the synthesis of **9ZASHPL2**, starting from **SHPL2** (10 mg, 11.2  $\mu$ mol), Boc-Pro-OH (13 mg, 57  $\mu$ mol), DIPCPI (5  $\mu$ l) and DCM (300  $\mu$ l), the title compound (9 mg, 74%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, gradient ACN/H<sub>2</sub>O 85:15-100:0 in 10 min (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 18.7 min).

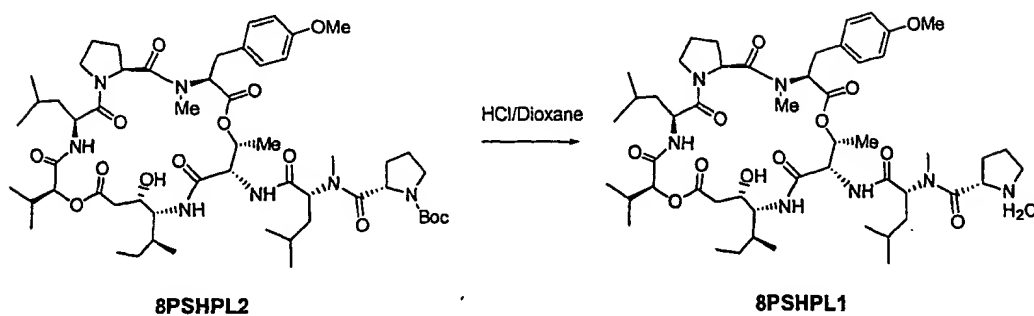
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.82-1.10 (m, 24H), 1.12-2.30 (m, 22H), 1.47 (s, 9H), 2.41 (m, 1H), 3.04 (s, 3H), 3.10-3.74 (m, 7H), 3.78 (s, 3H), 3.91 (m, 1H), 4.01 (m, 1H), 4.31 (m, 1H), 4.59 (m, 1H), 4.87 (m, 1H), 5.02 (d,  $J$  = 4.8, 1H), 5.16 (m, 1H), 5.36 (m, 1H), 6.84 (d,  $J$  = 8.3, 2H), 7.07 (d,  $J$  = 8.3, 2H), 7.17 (d,  $J$  = 6.3, 1H), 7.35 (d,  $J$  = 9.7, 1H), 7.85 (d,  $J$  = 9.7, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  12.04, 14.41, 16.76, 17.97, 19.12, 21.03, 21.66, 23.92, 24.00, 24.77, 25.09, 25.20, 27.53, 28.21, 28.72, 29.67, 30.35, 31.23, 33.80, 34.30, 36.24, 39.05, 39.37, 39.80, 46.90, 47.33, 48.42, 54.48, 55.49, 55.58, 55.84, 57.12, 58.08, 66.31, 69.11, 71.05, 79.23, 80.28, 114.29, 130.23, 130.59, 154.91, 158.82, 168.37, 169.88, 170.66, 170.86, 171.28, 171.40, 174.10, 174.79.

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ESI-MS Calcd for  $C_{56}H_{91}N_7O_{14}$ : 1085.6. Found  $m/z$ : 1086.7  
( $M+H$ )<sup>+</sup>.

## Example 105

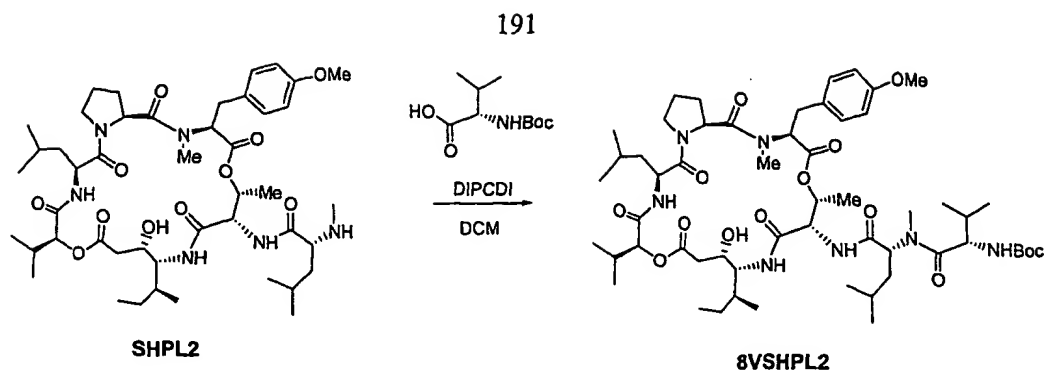
Synthesis of [Hiv]<sup>3</sup>-[Pro]<sup>8</sup>-didemnin A (8PSHPL1)

Following the procedure described for the synthesis of **9ASHPL1**, starting from **8PSHPL2** (7 mg, 6.4  $\mu$ mol), the title compound (6 mg, quant.) was obtained as a white solid.

ESI-MS Calcd for  $C_{51}H_{81}N_7O_{12}$ : 983.59. Found  $m/z$ : 984.6 ( $M+H$ )<sup>+</sup>.

## Example 106

Synthesis of [Hiv]<sup>3</sup>-[Boc-Val]<sup>8</sup>-didemnin A (8VSHPL2)



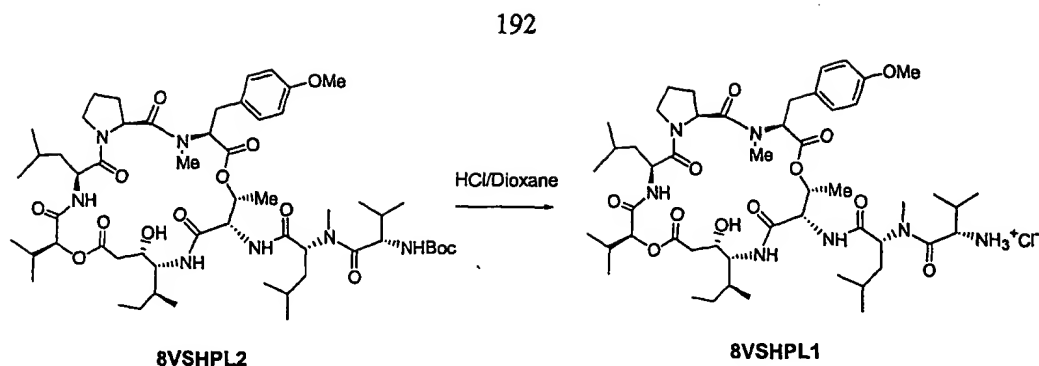
Following the procedure described for the synthesis of **92ASHPL2**, starting from **SHPL2** (10 mg, 11.2  $\mu$ mol), Boc-Val-OH (12 mg, 56  $\mu$ mol), DIPCPI (5  $\mu$ l) and DCM (300  $\mu$ l), the title compound (9 mg, 82%) was obtained as a white solid after purification by HPLC (HyperPrep PEP 100 C18, gradient ACN/H<sub>2</sub>O 85:15-100:0 in 10 min (flow: 7 ml/min, 250x21 mm, at 270 nm,  $t_R$  = 22.1 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.82-1.10 (m, 30H), 1.12-1.82 (m, 12H), 1.45 (s, 9H), 1.84-2.40 (m, 6H), 2.56 (s, 3H), 2.96 (s, 3H), 2.97 (m, 3H), 3.13 (m, 1H), 3.35 (m, 1H), 3.56 (m, 1H), 3.65 (m, 2H), 3.79 (s, 3H), 3.85 (m, 1H), 4.03 (m, 1H), 4.24 (m, 1H), 4.41 (m, 1H), 4.61 (m, 1H), 4.88 (m, 1H), 4.99 (d,  $J_1$  = 5.3, 1H), 5.07 (m, 1H), 5.19 (m, 1H), 5.60 (d,  $J$  = 8.3, 1H), 6.84 (d,  $J$  = 8.3, 2H), 7.03 (d,  $J$  = 8.7, 2H), 7.07 (d,  $J$  = 8.3, 2H), 7.46 (d,  $J$  = 10.2, 1H), 7.84 (d,  $J$  = 9.2, 1H).

ESI-MS Calcd for C<sub>56</sub>H<sub>91</sub>N<sub>7</sub>O<sub>14</sub>: 1085.6. Found  $m/z$ : 1086.7 (M+H)<sup>+</sup>.

#### Example 107

#### Synthesis of [Hiv]<sup>3</sup>-[Val]<sup>8</sup>-didemnin A (8VSHPL1)

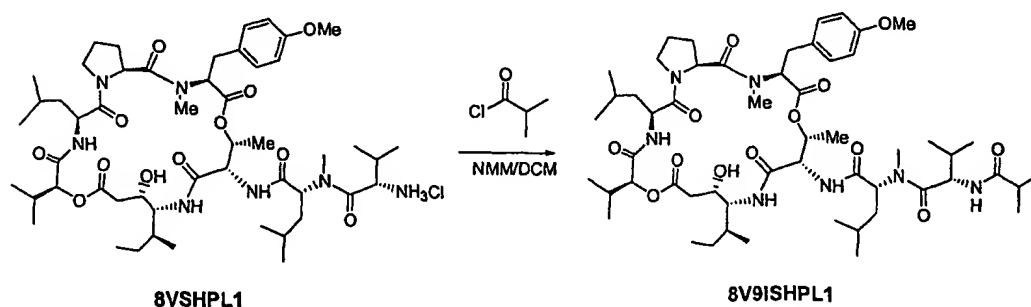


Following the procedure described for the synthesis of **9ASHPL1**, starting from **8VSHPL2** (8 mg, 7.4  $\mu$ mol), the title compound (7 mg, quant.) was obtained as a white solid.

ESI-MS Calcd for  $C_{51}H_{83}N_7O_{12}$ : 985.61. Found: (m/z): 986.6 (M+H)<sup>+</sup>.

### Example 108

### Synthesis of [Hiv]<sup>3</sup>-[Val]<sup>8</sup>-[Isobutyryl]<sup>9</sup>-didemnin A (8V9ISHPL1)



To a solution of **8VSHPL1** (6 mg, 5.8  $\mu$ mol) in DCM (200  $\mu$ ml ) at 0° C under Ar, were added NMM (3.3  $\mu$ l, 30  $\mu$ mol) and isobutyryl chloride (2  $\mu$ l, 19  $\mu$ mol). After 5 h of stirring at r.t., the reaction mixture

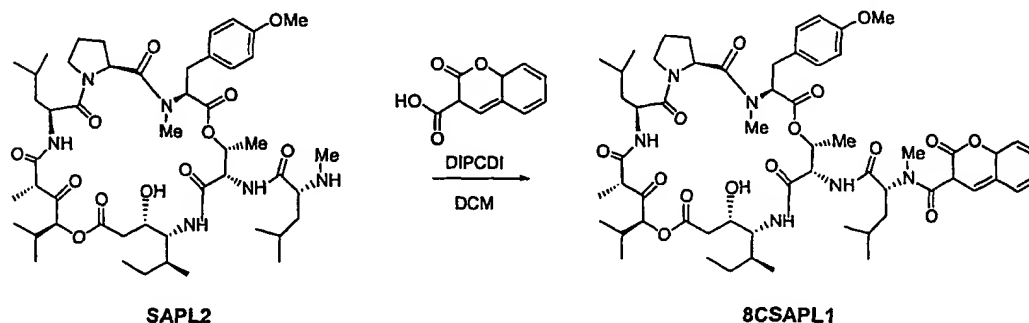
was diluted with DCM (5 ml) and washed successively with aq.  $\text{KHSO}_4$  (5 ml, 10%), aq.  $\text{HCO}_3\text{Na}$  (5 ml, sat) and brine (5 ml). The organic solution was dried ( $\text{Na}_2\text{SO}_4$ ), filtered and concentrated at reduced pressure to yield **8V9ISHPL1** (6 mg, 97%) as a white solid.

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.82-1.10 (m, 36H), 1.12-1.85 (m, 10H), 1.90-2.45 (m, 6H), 2.56 (s, 3H), 2.90 (m, 1H), 3.00 (s, 3H), 3.13 (m, 2H), 3.36 (dd,  $J_1=4.3$ ,  $J_2=14.1$ , 1H), 3.64 (m, 6H), 3.78 (s, 3H), 3.87 (m, 1H), 3.97 (m, 1H), 4.21 (m, 1H), 4.34 (m, 2H), 4.61 (m, 1H), 4.87 (m, 1H), 4.98 (d,  $J=4.8$ , 1H), 5.17 (m, 2H), 6.24 (d,  $J=6.3$ , 1H), 6.84 (d,  $J=8.3$ , 2H), 7.07 (d,  $J=8.3$ , 2H), 7.32 (d,  $J=6.3$ , 1H), 7.35 (d,  $J=4.3$ , 1H), 7.81 (d,  $J=9.7$ , 1H).

ESI-MS Calcd for  $\text{C}_{55}\text{H}_{89}\text{N}_7\text{O}_{13}$ , 1055.6. Found  $m/z$ : 1056.7 ( $\text{M}+\text{H}$ ) $^+$ .

#### Example 109

##### Synthesis of [coumarin] $^8$ -didemnin A (**8CSAPL1**)



Following the procedure described for the synthesis of **9ZASHPL2**, starting from **SAPL2** (20 mg, 21  $\mu\text{mol}$ ) and coumarin-3-carboxylic acid (20 mg, 107  $\mu\text{mol}$ ), the title compound (18 mg, 76%) was obtained as a

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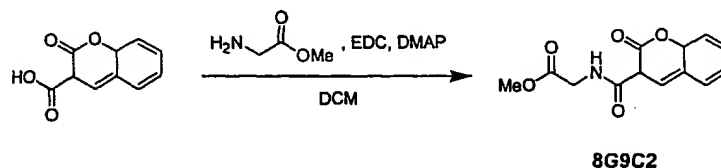
white solid after purification by HPLC (HyperPrep PEP 100 C18, isocratic ACN/H<sub>2</sub>O 85:15 (flow: 7 ml/min, 250x21 mm, at 270 nm, t<sub>R</sub> = 16.5 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.78-1.00 (m, 24H), 1.20-2.50 (m, 22H), 2.56 (s, 3H), 2.92 (s, 3H), 3.08-3.25 (m, 2H), 3.90 (m, 1H), 3.60 (m, 2H), 3.70 (m, 1H), 3.79 (s, 3H), 3.92-4.25 (m, 3H), 4.60 (m, 1H), 4.80 (m, 2H), 5.15 (m, 1H), 5.18 (d, J= 3.4, 1H), 5.28 (m, 1H), 6.84 (d, J= 8.3, 2H), 7.08 (d, J= 8.3, 2H), 7.20 (d, J= 6.3, 1H), 7.37 (m, 2H), 7.45 (d, J= 9.7, 1H), 7.58 (m, 2H), 7.96 (m, 1H), 8.23 (m, 1H).

ESI-MS Calcd for C<sub>59</sub>H<sub>82</sub>N<sub>6</sub>O<sub>15</sub>: 1114.58. Found: 1116.3(M+H)<sup>+</sup>.

#### Example 110

#### Synthesis of coumarin-3-carbonylamino-acetic acid methyl ester (8G9C2)



To a round bottom flask containing methyl-glycine (89 mg, 1.00 mmol), 3-carboxy coumarine, anh. DCM (25 ml), under Ar, N'-(3 Dimethylaminopropyl)-N-ethyl-carbodiimid hydrochlorid (EDC) (479 mg, 2.50 mmol) and DMAP (489 mg, 4.00 mmol) were added at room temperature. The resulting mixture was stirred for 1 h 30 min (total conversion was observed by TLC). Then, DCM (20 ml) was added and the solution was washed successively with aq. NaHCO<sub>3</sub> (10 ml, sat) and brine (10 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated. The resulting orange solid obtained was purified by flash

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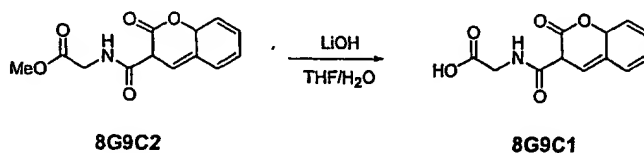
LC (silica gel, grad hex:EtOAc 1:1 to 2:1) to give the title compound (445 mg, quant) as a colourless oil.  $R_f = 0.08$  (Hex/EtOAc 1:1).

$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  3.79 (s, 3H), 4.25 (d,  $J = 5.4$ , 2H), 7.40 (m, 2H), , 7.74 (d,  $J = 7.3$ , 1H), 7.68 (m, 2H), 8.91 (s, 1H), 9.25 (s, 1H).

ESI-MS Calcd for  $\text{C}_{13}\text{H}_{11}\text{NO}_5$ : 261.06. Found: 283.1 ( $\text{M} + \text{Na}$ ) $^+$ .

#### Example 111

##### Synthesis of coumarin-3-carboxylamino-acetic acid (**8G9C1**)



A solution of coumarin-3-carboxylamino-acetic acid methyl ester (312 mg, 1.19 mmol), in THF (12 ml) under Ar atmosphere, at 0° C (ice bath), a solution of LiOH in H<sub>2</sub>O (0.2 M) was added dropwise. The reaction mixture was stirred vigorously at room temperature until total conversion was observed by TLC (2 hours). The solution was partially concentrated and Et<sub>2</sub>O was added (10 ml). The organic layer was washed with NaHCO<sub>3</sub> (10 ml, sat) and the combined aqueous layers were acidified with 10% KHSO<sub>4</sub> (pH = 3-4) and extracted with ether (3x20 ml). The organic layer was concentrated at reduced pressure to afford **8G9C1** (280 mg, 95%) as a white solid.

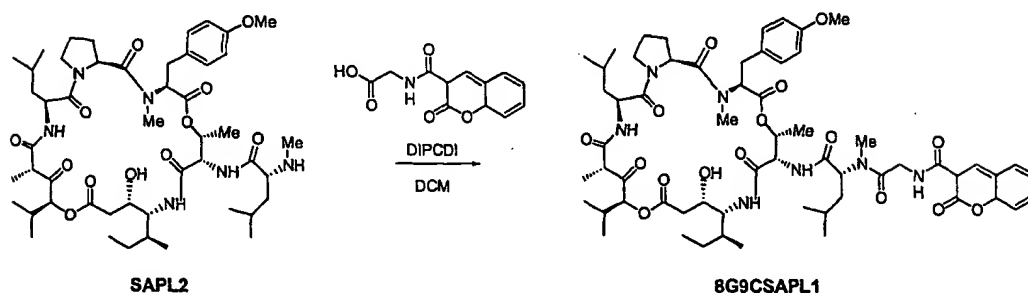
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  4.18 (d,  $J = 5.3$ , 2H), 7.41 (m, 1H), 7.45 (d,  $J = 7.8$ , 1H), 7.74 (d,  $J = 7.3$ , 1H), 7.78 (t,  $J = 8.3$ , 1H), 7.85 (d,  $J = 7.8$ , 1H), 8.89 (s, 1H), 9.41 (m, 1H).



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ESI-MS Calcd for  $C_{12}H_9NO_5$ : 247.05. Found: 248.0 (M+H)<sup>+</sup>.

## Example 112

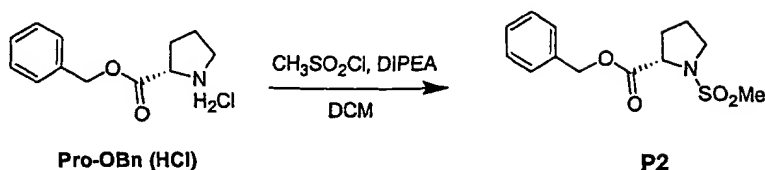
**Synthesis of [Gly]<sup>8</sup>-[coumarin]<sup>9</sup>-didemnin A (8G9CSAPL1)**

Following the procedure described for the synthesis of **9ZASHPL2**, starting from **SAPL2** (20 mg, 21  $\mu$ mol) and coumarin-3-carboxylic acid (26 mg, 105  $\mu$ mol), the title compound (18 mg, 72%) was obtained as a white solid after HPLC (Symetry Prep<sup>TM</sup> C18, isocratic ACN/H<sub>2</sub>O 60:40 (flow: 3 ml/min, 150x7.8 mm, at 270 nm,  $t_R$  = 17.5 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.85-0.94 (m, 24H), 1.23-2.17 (m, 21H), 2.30-2.44 (m, 1H), 2.56 (s, 3H), 3.01 (s, 3H), 3.10-3.24 (m, 2H), 3.40 (dd,  $J_1=5.4$ ,  $J_2=14.2$ , 1H), 3.59-3.74 (m, 3H), 3.79 (s, 3H), 3.99-4.21 (m, 4H), 4.37-4.44 (m, 1H), 4.60 (m, 1H), 4.68 (m, 1H), 4.81 (t,  $J=9.8$ , 1H), 5.18 (d,  $J=3.4$ , 1H), 5.25 (dd,  $J_1=2.9$ ,  $J_2=5.9$ , 1H), 5.20-5.45 (m, 1H), 6.84 (d,  $J=8.3$ , 2H), 7.08 (d,  $J=8.3$ , 2H), 7.19-7.28 (m, 1H), 7.34-7.42 (m, 2H), 7.63-7.72 (m, 2H), 7.88 (d,  $J=8.3$ , 2H), 9.00 (s, 1H), 9.57 (m, 1H).

ESI-MS Calcd for  $C_{61}H_{85}N_7O_{16}$ : 1171.61. Found: 1172.5 (M+H)<sup>+</sup>.

## Example 113

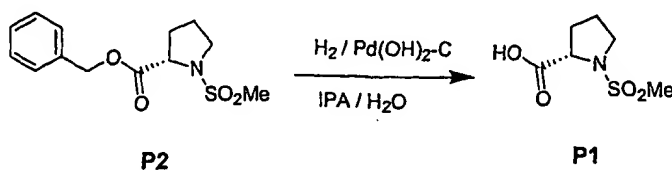
**Synthesis of N-methylsulphonyl-Pro-OBz (P2)**

To a solution of Pro-OBn (HCl) (300 mg, 1.24 mmol) in DCM (25 ml, anh) at 0°C under Ar, DIPEA (0.7 ml) and methanesulphonyl chloride (116 µl) were added dropwise by syringe. The reaction mixture was stirred at room temperature overnight. DCM (10 ml) was added and the solution was washed successively with aq. KHSO<sub>4</sub> (15 ml, 10%), aq. NaHCO<sub>3</sub> (15 ml, sat) and brine (15 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to reduce pressure to yield pure **P2** (350 mg, 1.24 mmol, quant) as a white solid. R<sub>f</sub> = 0.55 (Hex/AcOEt 1:1).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 1.91-2.08 (m, 3H), 2.19-2.31 (m, 1H), 2.93 (s, 3H), 3.38-3.54 (m, 2H), 3.65 (s, 3H), 4.51 (dd, J<sub>1</sub> = 3.4, J<sub>2</sub> = 8.3, 1H), 5.11 (d, J = 12.2, 1H), 5.18 (d, J = 12.2, 1H), 7.31 (m, 5H).

ESI-MS Calcd for C<sub>13</sub>H<sub>17</sub>NO<sub>4</sub>S: 283.09. Found: 284.1 (M+H)<sup>+</sup>.

## Example 114

**Synthesis of N-methylsulphonyl-Pro-OH (P1)**

A degassed mixture of N-methylsulphonyl-Pro-OBz (**P2**) (250 mg, 0.88 mmol) and Pd(OH)<sub>2</sub>/C (20% Pd, 100 mg, 40% w/w) in IPA:H<sub>2</sub>O (26 ml:13 ml), was saturated with H<sub>2</sub> and maintained at 1 atm of hydrogen gas while stirring for 3h. Then, the mixture was filtered through a Teflon filter (0.45  $\mu$ m), and concentrated under vacuum to yield the title compound (128 mg, 75% yield) as a white solid with no further purification.

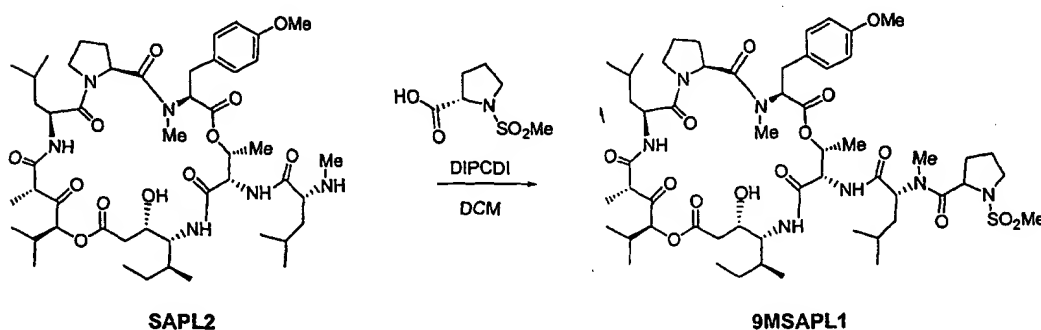
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  1.94-2.03 (m, 2H), 2.07-2.14 (m, 1H), 2.22-2.35 (m, 1H), 2.96 (s, 3H), 3.44 (m, 2H), 4.44 (dd, J<sub>1</sub>= 3.9, J<sub>2</sub>= 8.8, 2H), 9.10 (m, 1H).

<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  24.96, 31.08, 38.46, 48.06, 60.66, 174.75.

ESI-MS Calcd for C<sub>6</sub>H<sub>11</sub>NO<sub>4</sub>S: 193.22. Found: 194.0 (M+H)<sup>+</sup>.

#### Example 115

#### Synthesis of [Methylsulphonyl]<sup>9</sup>-aplidine (**9MSAPL1**)



Following the procedure described for the synthesis of **9ZASHPL2**, starting from **SAPL2** (10 mg, 10.7  $\mu$ mol) and N-methylsulphonyl-Pro-OH

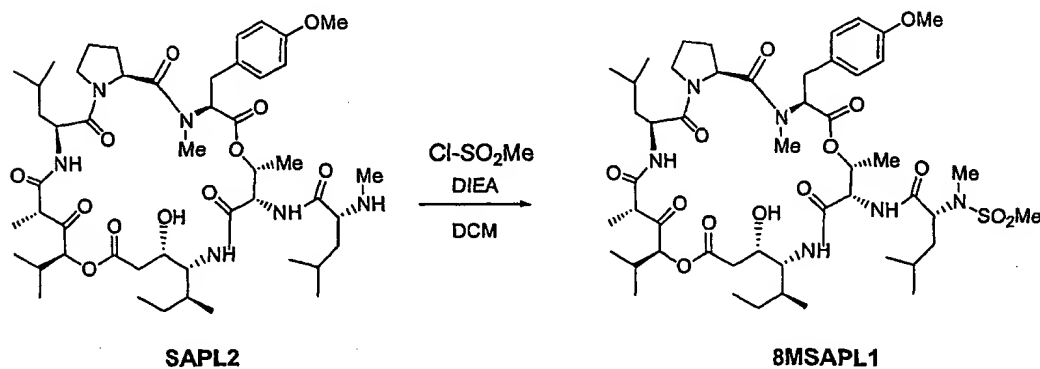
(10 mg, 53  $\mu$ mol), the title compound (9 mg, 74%) was obtained as a white solid after purification by HPLC (Symetry Prep C18, isocratic ACN/H<sub>2</sub>O 60:40 (flow: 3 ml/min, 150x7.8 mm, at 270 nm,  $t_R$  = 9 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.78-1.00 (m, 24H), 1.20-2.50 (m, 26H), 2.56 (s, 3H), 2.93 (m, 1H), 3.04 (s, 3H), 3.06 (s, 3H), 3.08-3.25 (m, 2H), 3.28-3.50 (m, 2H), 3.60 (m, 2H), 3.70 (m, 1H), 3.79 (s, 3H), 4.05 (m, 2H), 4.17 (m, 1H), 4.60 (m, 2H), 4.81 (m, 2H), 5.10 (m, 1H), 5.19 (d,  $J$ = 3.4, 1H), 5.33 (m, 1H), 6.84 (d,  $J$ = 8.3, 2H), 6.86 (m, 1H), 7.07 (d,  $J$ = 8.3, 2H), 7.09 (m, 1H), 7.82 (d,  $J$ = 9.2, 1H).

ESI-MS Calcd for C<sub>55</sub>H<sub>87</sub>N<sub>7</sub>O<sub>15</sub>S: 1117.60. Found: 1118.7 (M+H)<sup>+</sup>.

#### Example 116

#### Synthesis of [Methylsulphonyl]<sup>8</sup>-didemnin A (8MSAPL1)



To a solution of **SAPL2** (10 mg, 10.7  $\mu$ mol) in DCM (200  $\mu$ l, anh) at 0°C under Ar, were added DIPEA (3  $\mu$ l) and methanesulphonyl chloride (0.85  $\mu$ l). The reaction mixture was stirred at 5°C overnight. DCM (10 ml) was added and the solution was washed successively with aq. KHSO<sub>4</sub>

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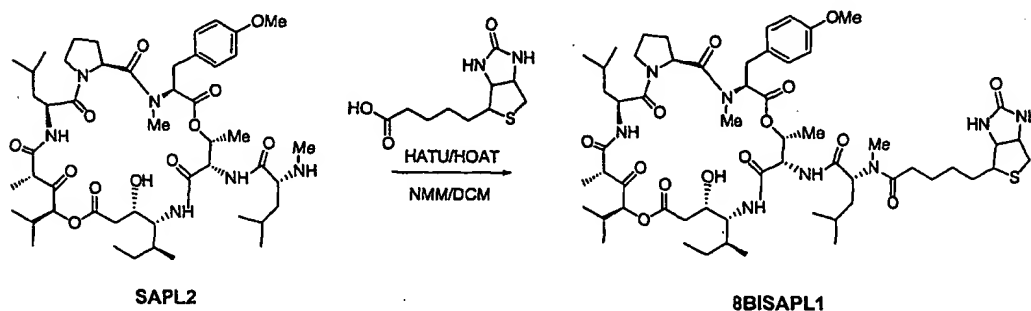
(5 ml, 10%), aq. NaHCO<sub>3</sub> (5 ml, sat) and brine (5 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to reduce pressure to yield pure **8MSAPL1** (11 mg, quant) as a white solid.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.83-0.97 (m, 24H), 1.10-1.45 (m, 10H), 1.50-1.65 (m, 5H), 1.76-1.82 (m, 2H), 2.00-2.20 (m, 3H), 2.27-2.36 (m, 1H), 2.45-2.55 (m, 1H), 2.56 (s, 3H), 2.90 (s, 3H), 3.02 (s, 3H), 3.08 (d, J=16.6, 1H), 3.17 (dd, J<sub>1</sub>=10.7, J<sub>2</sub>=14.1, 1H), 3.37 (m, 1H), 3.60 (m, 2H), 3.70 (m, 1H), 3.79 (s, 3H), 3.99-4.11 (m, 3H), 4.49 (m, 1H), 4.59 (m, 1H), 4.78 (m, 2H), 5.06 (m, 1H), 5.19 (d, J=3.9, 1H), 6.68 (d, J=8.8, 1H), 6.84 (d, J=8.3, 2H), 7.07 (d, J=8.3, 2H), 7.33 (d, J=9.8, 1H), 7.61 (d, J=8.8, 1H).

ESI-MS Calcd for C<sub>50</sub>H<sub>80</sub>N<sub>6</sub>O<sub>14</sub>S: 1020.55. Found: 1022.1 (M+H)<sup>+</sup>.

#### Example 117

#### Synthesis of [Biotin]<sup>8</sup>-didemnin A (**8BISAPL1**)



To a solution of HATU (24 mg, 61 μmol), HOAt (8 mg, 63 μmol), **SAPL2** (20 mg, 21.4 μmol) and d-Biotin (7.8 mg, 32 μmol), in anh. DCM (400 μL) at 0 °C under Ar, NMM was added dropwise by syringe. The

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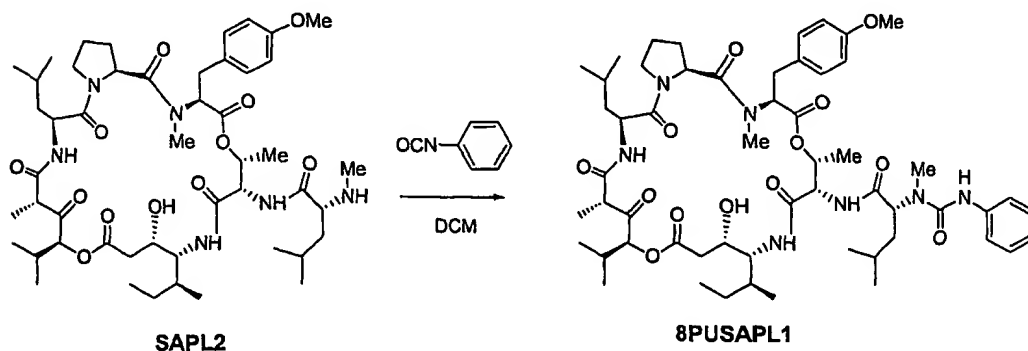
resulting mixture was stirred for 2 h at 0°C and then, at room temperature for additional 14 h. DCM (10 ml) was added and the solution was washed successively with aq. KHSO<sub>4</sub> (5 ml, 10%), aq. NaHCO<sub>3</sub> (5 ml, sat.) and brine (5ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated under reduced pressure. The title compound (18 mg, 72%) was obtained as a white solid after purification by HPLC (Symetry Prep C18, gradient ACN/H<sub>2</sub>O 60:40-100:0 in 10 min. (flow: 3 ml/min, 150x7.8 mm, at 270 nm, t<sub>R</sub> = 6 min).

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 0.78-1.00 (m, 24H), 1.20-1.98 (m, 23H), 2.00-2.62 (m, 7H), 2.53 (s, 3H), 2.80-3.00 (m, 2H), 2.86 (s, 3H), 3.16 (m, 2H), 3.35 (m, 2H), 3.57 (m, 2H), 3.70 (m, 1H), 3.79 (s, 3H), 4.01 (m, 2H), 4.10 (m, 1H), 4.31 (m, 1H), 4.46 (m, 1H), 4.56 (m, 1H), 4.82 (m, 2H), 5.00 (m, 1H), 5.14 (m, 2H), 5.67 (s, 1H), 6.23 (s, 1H), 6.84 (d, J= 8.3, 2H), 7.07 (d, J= 8.3, 2H), 7.28 (d, J= 8.5, 1H), 7.35 (d, J= 7.8, 1H), 8.01 (d, J= 8.7, 1H).

ESI-MS Calcd for C<sub>59</sub>H<sub>92</sub>N<sub>8</sub>O<sub>14</sub>S: 1168.65. Found: 1169.6 (M+H)<sup>+</sup>.

Example 118

#### Synthesis of [Phenylurea]<sup>8</sup>-didemnin A (8PUSAPL1)



To a solution of **SAPL2** (10 mg, 10.7  $\mu$ mol) in DCM (200  $\mu$ l, anh) at 0°C under Ar, phenyl isocyanate (1.3  $\mu$ l, 12  $\mu$ mol) was added and the reaction mixture was stirred at r.t. for 4 hours. DCM (10 ml) was added and the solution was washed successively with aq. KHSO<sub>4</sub> (5 ml, 10%), aq. NaHCO<sub>3</sub> (5 ml, sat) and brine (5 ml). The organic phase was dried (Na<sub>2</sub>SO<sub>4</sub>), filtered and concentrated to reduced pressure to yield pure **8PUSAPL1** (11 mg, 94%) as a white solid.

<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.85-0.94 (m, 24H), 1.11-1.43 (m, 4H), 1.22 (d, J=6.8, 3H), 1.35 (d, J=6.8, 3H), 1.49-1.83 (m, 5H), 1.73 (s, 3H), 2.02 (m, 1H), 2.14 (m, 2H), 2.34 (dt, J<sub>1</sub>=3.4, J<sub>2</sub>=6.8, 1H), 2.54 (s, 3H), 2.91 (s, 3H), 3.04 (d, J=16.6, 1H), 3.17 (dd, J<sub>1</sub>=11.2, J<sub>2</sub>=14.6, 1H), 3.36 (dd, J<sub>1</sub>=3.9, J<sub>2</sub>=14.2, 1H), 3.57 (dd, J<sub>1</sub>=4.4, J<sub>2</sub>=10.7, 1H), 3.59-3.63 (m, 1H), 3.67-3.75 (m, 1H), 3.79 (s, 3H), 3.96-4.10 (m, 2H), 4.19 (q, J=6.8, 1H), 4.58 (m, 1H), 4.74 (dd, J<sub>1</sub>=3.9, J<sub>2</sub>=8.8, 1H), 4.78-4.85 (m, 1H), 5.05 (m, 2H), 5.17 (d, J=3.4, 1H), 6.52 (s, 1H), 6.84 (d, J=8.3, 2H), 7.03-7.09 (m, 3H), 7.22-7.32 (m, 4H), 7.39 (d, J=8.3, 2H), 7.93 (d, J=8.8, 1H).

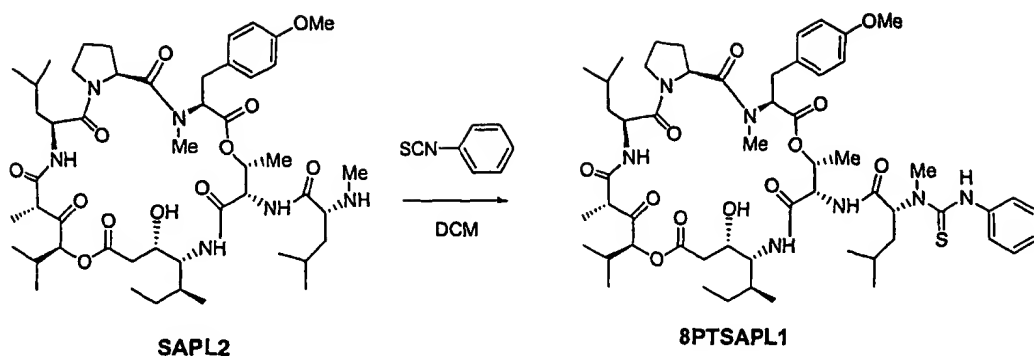
<sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  11.70, 15.51, 17.16, 18.74, 21.11, 22.41, 23.29, 23.98, 24.94, 25.10, 25.32, 26.97, 28.18, 30.36, 31.57, 34.44, 36.51, 38.86, 41.64, 45.23, 47.30, 49.80, 50.08, 54.60, 55.50, 56.06, 57.51, 62.77, 66.45, 68.17, 70.72, 81.93, 114.36, 120.76, 123.86, 129.12, 130.04, 130.57, 138.78, 141.00, 157.78, 158.87, 169.92, 170.68, 171.49, 172.43, 173.44, 181.47, 192.38, 204.93, 206.66.

ESI-MS Calcd for C<sub>56</sub>H<sub>83</sub>N<sub>7</sub>O<sub>13</sub>: 1161.60. Found: 1062.6 (M+H)<sup>+</sup>.

#### Example 119

#### Synthesis of [Pheylthiourea]<sup>8</sup>-didemnin A (**8PTSAPL1**)

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Following the procedure for the synthesis of **8PUSAPL1**, starting from **SAPL2** (10 mg, 10.7  $\mu$ mol) and phenyl thioisocyanate (1.3  $\mu$ l, 12  $\mu$ mol), after 15 h of stirring the title compound (11 mg, 93%) was obtained as a white solid with no further purification.

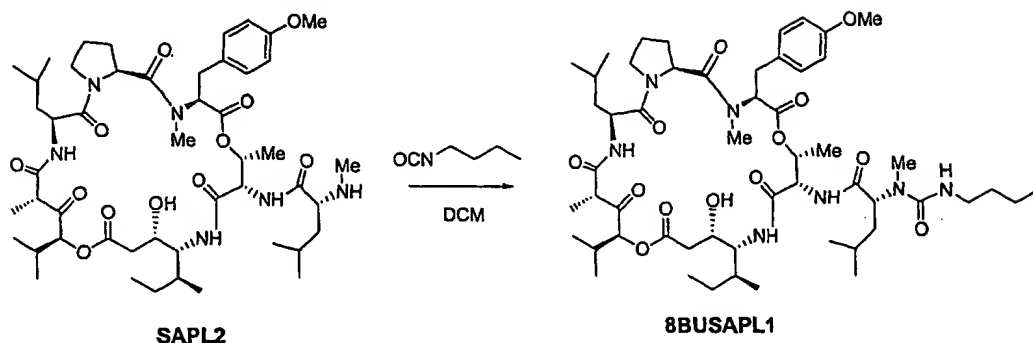
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.84-0.99 (m, 24H), 1.14-1.46 (m, 9H), 1.46-1.78 (m, 9H), 1.98-2.19 (m, 3H), 2.34 (m, 1H), 2.54 (s, 3H), 2.91 (m, 1H), 3.00 (s, 3H), 3.08-3.21 (m, 1H), 3.36 (dd,  $J_1=4.4$ ,  $J_2=14.1$ , 1H), 3.55-3.64 (m, 2H), 3.66-3.74 (m, 1H), 3.79 (s, 3H), 3.96-4.12 (m, 2H), 4.21 (q,  $J=6.8$ , 1H), 4.59 (t,  $J=5.3$ , 1H), 4.75 (dd,  $J_1=3.4$ ,  $J_2=8.3$ , 1H), 4.83 (t,  $J=10.3$ , 1H), 5.05-5.12 (m, 2H), 5.16 (d,  $J=3.4$ , 1H), 6.30 (t,  $J=7.3$ , 1H), 6.84 (d,  $J=8.3$ , 2H), 7.07 (d,  $J=8.3$ , 2H), 7.21-7.37 (m, 6H), 7.93 (d,  $J=8.8$ , 1H), 8.08 (d,  $J=8.8$ , 1H).

$^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  11.68, 15.28, 15.85, 17.15, 18.73, 21.12, 22.73, 23.66, 24.01, 25.13, 25.33, 26.62, 28.16, 29.92, 31.53, 32.42, 32.90, 34.41, 36.77, 38.86, 41.69, 47.28, 50.12, 55.50, 56.48, 57.51, 59.87, 66.47, 70.75, 81.91, 114.37, 125.25, 125.95, 126.57, 127.54, 129.12, 129.77, 130.06, 130.57, 135.43, 158.88, 168.54, 169.90, 170.70, 171.49, 172.38, 190.41.

ESI-MS Calcd for  $\text{C}_{56}\text{H}_{83}\text{N}_7\text{O}_{12}\text{S}$ : 1077.58. Found: 1078.5 ( $\text{M}+\text{H}$ ) $^+$ .



## Example 120

**Synthesis of [Butylurea]<sup>8</sup>-didemnin A (8BUSAPL1)**

Following the procedure for the synthesis of **8PUSAPL1**, starting from **SAPL2** (10 mg, 10.7  $\mu\text{mol}$ ) and butyl thioisocyanate (1.4  $\mu\text{l}$ , 12  $\mu\text{mol}$ ), after 4 h of stirring the title compound (9 mg, 78%) was obtained as a white solid with no further purification.

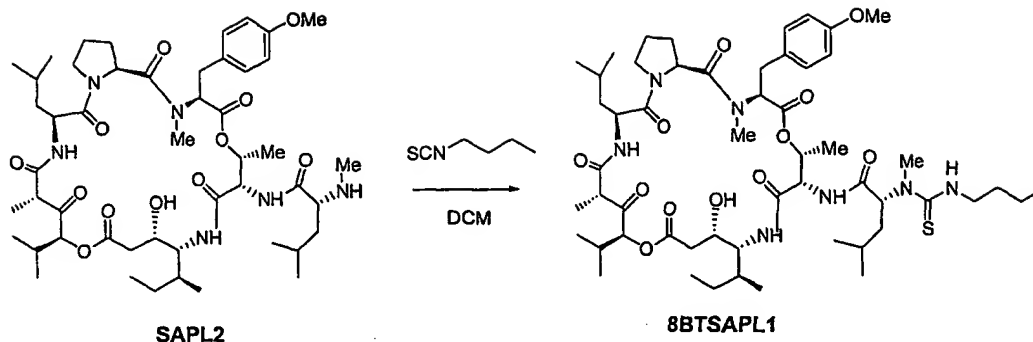
$^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  0.85-0.94 (m, 24H), 1.10-1.80 (m, 24H), 2.03 (m, 1 H), 2.13 (m, 2H), 2.33 (m, 1H), 2.55 (s, 3H), 2.72 (s, 3H), 3.02 (d,  $J=16.1$ , 1H), 3.17 (dd,  $J_1=11.2$ ,  $J_2=14.2$ , 1H), 3.23-3.40 (m, 3H), 3.62-3.78 (m, 3H), 3.79 (s, 3H), 4.03 (m, 2H), 4.19 (q,  $J=6.8$ , 1H), 4.58 (m, 2H), 4.71 (dd,  $J_1=3.4$ ,  $J_2=8.3$ , 1H), 4.82 (m, 1H), 5.00 (m, 2H), 5.16 (d,  $J=3.4$ , 1H), 5.22-5.28 (m, 2 H), 6.85 (d,  $J=8.8$ , 2H), 7.08 (d,  $J=8.3$ , 2H), 7.21-7.29 (m, 1H), 7.96 (d,  $J=9.2$ , 1H).

ESI-MS Calcd for  $\text{C}_{54}\text{H}_{87}\text{N}_7\text{O}_{13}$ : 1041.64. Found: 1042.7 ( $\text{M}+\text{H}$ )<sup>+</sup>.

## Example 121

**Synthesis of [Butylthiourea]<sup>8</sup>-didemnin A (8BTSAPL1)**

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Following the procedure for the synthesis of **8PUSAPL1**, starting from **SAPL2** (10 mg, 10.7  $\mu$ mol) and butyl thioisocyanate (1.5  $\mu$ l, 12  $\mu$ mol), after 15 h of reaction the title compound (10 mg, 86%) was obtained as a white solid with no further purification.

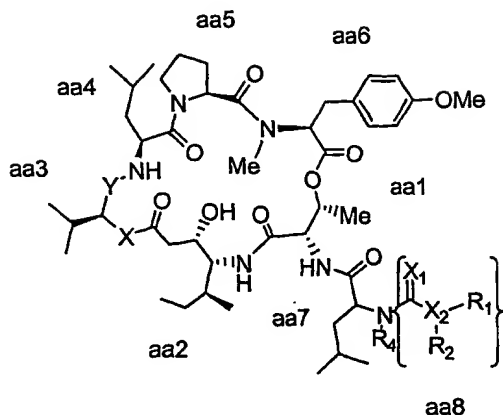
<sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  0.86-0.97 (m, 24H), 1.14-1.78 (m, 27H), 1.99-2.14 (m, 3H), 2.34 (m, 1H), 2.55 (s, 3H), 2.88 (s, 3H), 3.01 (d, J= 16.6, 1 H), 3.16 (dd, J<sub>1</sub>=11.2, J<sub>2</sub>= 14.6, 1H), 3.36 (dd, J<sub>1</sub>=4.4, J<sub>2</sub>= 14.2, 1H), 3.49-3.74 (m, 3H), 3.79 (s, 3H), 4.02 (d, J= 6.8, 2H), 4.21 (q, J=6.8, 1H), 4.58 (m, 1H), 4.70 (dd, J<sub>1</sub>=2.9, J<sub>2</sub>= 8.3, 1H), 4.82 (t, J=10.3, 1H), 5.07-5.12 (m, 2H), 5.09 (d, J= 3.9, 1H), 5.60 (m, 1H), 6.36 (dd, J<sub>1</sub>=5.4, J<sub>2</sub>= 8.3, 1H), 6.84 (d, J=8.8, 2H), 7.08 (d, J=8.8, 2H), 7.84 (d, J= 8.3, 1H), 7.91 (d, J= 9.3, 1H).

ESI-MS Calcd for C<sub>54</sub>H<sub>87</sub>N<sub>7</sub>O<sub>12</sub>S: 1057.61. Found: 1080.7 (M+Na)<sup>+</sup>.

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## CLAIMS

1. A compound of the formula:



wherein:

X is independently -CR<sub>2</sub>-, -O-, -S-, or -NR-, in which R is independently H or an organic group selected from an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and their substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group;

X<sub>2</sub> is independently CR, O (and R<sub>2</sub> is absent), S (and R<sub>2</sub> is absent), or N, in which R is independently H or an organic group selected from an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and their substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group;

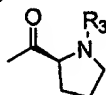
Y is -(COR')<sub>n</sub>CO-, where n is 0 or 1 and R' is an organic group selected from an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and their substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto

group, an optionally protected amino group, a guanidino group, or a halogen group;

X<sub>1</sub> is O or S;

R<sub>1</sub>, R<sub>2</sub> and R<sub>4</sub> are each independently H or an organic group selected from an amido group RCONH- or an acyl group RCO- where R is as defined, an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a heterocyclic group, an alkoxy group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group, and R<sub>1</sub> or R<sub>2</sub> when X<sub>2</sub> is N, and R<sub>4</sub>, can further be -SO<sub>2</sub>R, where R is as defined;

or R<sub>1</sub> and R<sub>2</sub> with X<sub>2</sub> may form an optionally N-substituted proline, the N-substituted proline **aa8** being of formula



where R<sub>3</sub> is independently H or an organic group selected from a group RSO<sub>2</sub>- or an acyl group RCO-, where R is as defined, or R<sub>3</sub> is an alkyl group, an alkenyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a carbonyl group, an hydroxy group, a mercapto group, an optionally protected amino group, a guanidino group, or a halogen group;

or R<sub>1</sub> and R<sub>2</sub> with X<sub>2</sub> may form a cycloalkyl, aryl or heterocyclic group, optionally substituted with one or more groups R<sub>3</sub>;

or R<sub>1</sub>, R<sub>2</sub>, X<sub>2</sub>, R<sub>4</sub> and the nitrogen bearing R<sub>4</sub> may form an oxadiazaspiroalkane N-substituted with R<sub>5</sub>, where R<sub>5</sub> is independently H or an organic group selected from a group RSO<sub>2</sub>- or an acyl group RCO where R is as defined, an alkyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a carbonyl group, an alkoxy group, an hydroxy group, a mercapto group, an amino group, a guanidino group, or a halogen group;

or **aa8** is replaced by an organic group selected from a group  $\text{RSO}_2$ - or an acyl group  $\text{RCO}$  where R is as defined, an alkyl group, an aryl group, an aralkyl group, and substituted derivatives substituted with one or more of a carbonyl group, an alkoxy group, an hydroxy group, a mercapto group, an amino group, a guanidino group, or a halogen group; and pharmaceutically acceptable salts thereof.

2. A compound according to claim 1, wherein X is  $\text{-NR-}$ , in which R is as defined.
3. A compound according to claim 2, wherein X is  $\text{-NH-}$  or  $\text{-NMe-}$ .
4. A compound according to claim 3, wherein X is  $\text{-NH-}$ .
5. A compound according to claim 1, wherein X is  $\text{-O-}$ .
6. A compound according to any preceding claim, wherein Y is  $\text{-COR'CO-}$ , where R' is an alkyl group.
7. A compound according to claim 6, wherein R' is  $\text{-CHCH}_3$ .
8. A compound according to any of claims 1 to 5, wherein Y is  $\text{-CO-}$ .

9. A compound according to claim 1, wherein X is --NH- or -O- and Y is -COCHCH<sub>3</sub>CO- or -CO-.
10. A compound according to any preceding claim, wherein R<sub>4</sub> is methyl.
11. A compound according to any preceding claim, wherein X<sub>1</sub> is =O.
12. A compound according to any preceding claim, wherein X<sub>2</sub>R<sub>1</sub> is an optionally substituted aralkyloxy group.
13. A compound according to claim 12, wherein X<sub>2</sub>R<sub>1</sub> is a benzyloxy group.
14. A compound according to any of claims 1 to 11, wherein X<sub>2</sub>R<sub>1</sub> is an optionally substituted amino group.
15. A compound according to claim 12, wherein X<sub>2</sub>R<sub>1</sub> is a group -NHR<sub>1</sub>, where R<sub>1</sub> is an optionally substituted alkyl group, alkenyl group, aryl group, or aralkyl group.
16. A compound according to claim 15, wherein R<sub>1</sub> is an alkyl group or an aryl group.

17. A compound according to claim 16, wherein  $R_1$  is a phenyl group or a butyl group.

18. A compound according to any of claims 1 to 11, wherein  $X_2R_1$  is an optionally substituted alkyl group.

19. A compound according to claim 18, where  $X_2R_1$  is a propyl group, isopropyl group, pentyl group or biotin group.

20. A compound according to any of claims 1 to 11, wherein - $C(=X_2)R_1R_2$  form an optionally substituted amino acid acyl group.

21. A compound according to claim 20, wherein the optionally substituted amino acid acyl group is optionally substituted proline or optionally substituted glycine or optionally substituted valine.

22. A compound according to claim 21, wherein the optionally substituted proline is optionally substituted norvaline-proline, optionally substituted alanine-proline, Boc-proline, optionally substituted alkyl-proline, or the optionally substituted glycine is heterocyclic-substituted glycine, or the optionally substituted valine is valine, Boc-valine, or alkyl-valine.

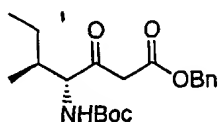
23. A compound according to claim 22, wherein the optionally substituted proline is norvaline-proline, Z-norvaline-proline, alanine-proline, Z-alanine-proline, Boc-alanine-proline, isobutyrylproline or optionally protected D-lactylproline, or the heterocyclic-substituted glycine is coumarinyl-glycine, or the optionally substituted valine is valine, Boc-valine, or isobutyrylvaline.
24. A compound according to any of claims 1 to 10, wherein  $X_1$  is S and  $X_2R_1$  is a group  $-NHR_1$ , where  $R_1$  is an optionally substituted alkyl group, alkenyl group, aryl group, or aralkyl group.
25. A compound according to claim 24, wherein  $R_1$  is an alkyl group or an aryl group.
26. A compound according to claim 25, wherein  $R_1$  is a phenyl group or a butyl group.
27. A compound according to any of claims 1 to 11, wherein  $R_1$  and  $R_2$  with  $X_2$  form a heterocyclic group, optionally substituted with one or more groups  $R_3$ .
28. A compound according to claim 27, wherein the heterocyclic group is coumarin.



29. A compound according to any of claims 1 to 10, wherein **aa8** is replaced by an organic group  $\text{RSO}_2$ -, where R is as defined.
30. A compound according to claim 29, wherein R is methyl.
31. A compound according to any of claims 1 to 10, wherein  $\text{R}_1$ ,  $\text{R}_2$ ,  $\text{X}_2$ ,  $\text{R}_4$  and the nitrogen bearing  $\text{R}_4$  form an oxadiazaspiroalkane N-substituted with  $\text{R}_5$ , where  $\text{R}_5$  is H.
32. A compound according to claim 31, wherein the N-substituted oxadiazaspiroalkane is 6-oxa-1,7-diazaspiro[4,4]nonane.
33. A compound according to claim 1, which is selected from:
- 3-[Aip]-Z-didemnin A,
  - 8-[Phenylurea]-didemnin A,
  - 8-[Butylurea]-didemnin A,
  - 3-[val]-8-[isobutyryl]-aplidine,
  - 9-[norvaline]-aplidine,
  - 3-[Hiv]-9-[Isobutyryl]-aplidine,
  - 3-[Val]-9-[Isobutyryl]-aplidine,
  - 3-[hiv]-8-[isobutyryl]-didemnin A,
  - 3-[Hiv]-9-[Ala]-aplidine,
  - 3-[Hiv]-9-[Nva]-aplidine,
  - 8-[Phenylthiourea]-didemnin A,
  - 8-[Coumarin]-didemnin A,
  - 8-[Butylthiourea]-didemnin A,
  - 3-[Hiv]-9-[D-Lac]-aplidine ,

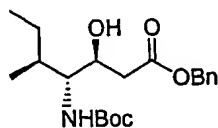
8-[Methylsulphonyl]-didemnin A,  
3-[val]-Z-didemnin A,  
3-[Hiv]-8-[Val]-didemnin A,  
3-[Hiv]-8-[butyryl]-aplidine,  
3-[val]-didemnin A,  
3-[Hiv]-didemnin A,  
Z-Didemnin A,  
9-[Z-Nva]-aplidine,  
3-[Hiv]-9-[Z-ala]-aplidine,  
8-[Gly]-9-[Coumarin]-didemnin A,  
8-[Biotin]-didemnin A,  
3-[Hiv]-7,8-[Spiro]-9-[Boc]-aplidine,  
3-[Hiv]-Z-didemnin A,  
3-[Hiv]-9-[Z-Nva]-aplidine,  
7,8-[Spiro]-9-[pyr]-aplidine,  
3-[Hiv]-9-[lac(OTBDMS)]-aplidine,  
3-[Hiv]-9-[Boc-Ala]-aplidine,  
7,8-[Spiro]-9-[Boc]-aplidine,  
3-[Hiv]-8-[Boc-Val]-aplidine,  
8-[Val]-9-[Isobutyryl]-didemnin A,  
3-[Hiv]-8-[hexanoyl]-didemnin A,  
3-[Val]-9-[Lac(OTBDMS)]-aplidine,  
3-[Aip]-didemnin A,  
3-[Hiv]-9-[D-Lac(OTBDMS)]-aplidine,  
7,8-[Spiro]-9-[Isobutyryl]-aplidine,  
3-[Hiv]-7,8-[Spiro]-9-[Pyr]-aplidine,  
3-[Hiv]-7,8-[Spiro]-9-[Isobutyryl]-aplidine,  
3-[Hiv]-7,8-[Spiro]-9-[Acryloyl]-aplidine, or  
[Aip]<sup>3</sup>-aplidine.

34. A compound according to any preceding claim, in the form a pharmaceutically acceptable salt.
35. A method of making a didemninn fragment having the structure

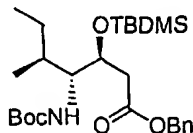


the method comprising coupling Boc-D-allo-Ileu-OH with the lithium enolate of benzyl acetate.

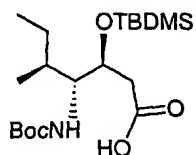
36. The method of claim 35, further reduction of the carbonyl group to yield a didemninn fragment having the structure



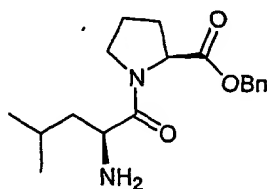
37. The method of claim 36, further protection of the hydroxyl group to yield a didemninn fragment having the structure



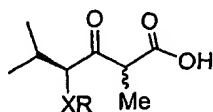
38. The method of claim 37, further deprotection of the benzyl ester group to yield a didemninn fragment having the structure



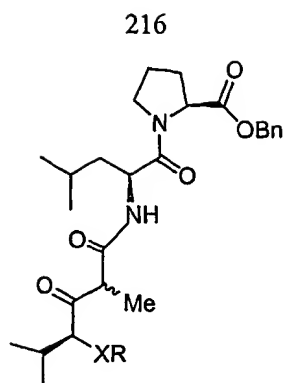
39. A method of making a didemninn fragment, the method comprising coupling a first reactant having the structure



and a second reactant having the structure

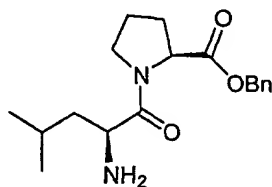


to yield a didemninn fragment having the structure



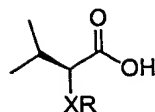
wherein X is selected from the group consisting of -O-, and -NH-; where R is an amine protecting group; and where R is a hydroxy protecting group.

40. The method of claim 39, wherein X is -O- and R is tert-butyldimethylsilyl
41. The method of claim 39, wherein X is -NH- and R is Boc
42. A method of making a didemnin fragment, the method comprising coupling a first reactant having the structure

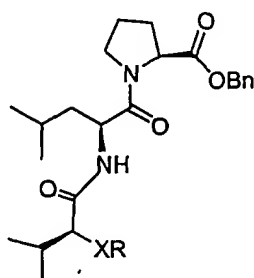


and a second reactant having the structure

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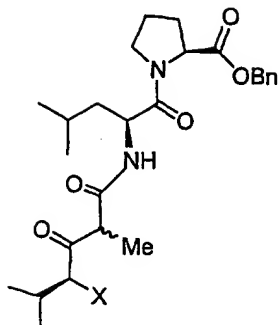
to yield a didemnin fragment having the structure



wherein X is selected from the group consisting of -O-, -NMe, and -NH-;  
where R is an amine protecting group; and where R is H.

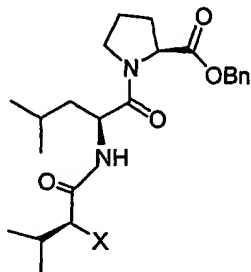
43. The method of claim 42, wherein X is -O- and R is H.
44. The method of claim 42, wherein X is -NH- and R is Boc.
45. The method of claim 42, wherein X is -NMe- and R is Boc.
46. The method of claim 39 to 41, further comprising hydrolyzing the didemnin fragment to yield a didemnin fragment having the structure

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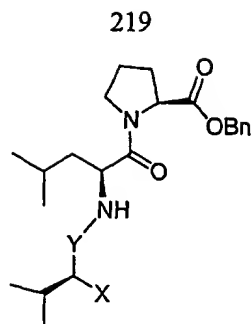
wherein X is selected from the group consisting of  $-OH$ , and  $-NH_2$

47. The method of claim 42 to 45, further comprising hydrolyzing the didemnin fragment to yield a didemnin fragment having the structure

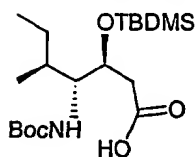


wherein X is selected from the group consisting of  $-NH_2$  and  $-NHMe$ .

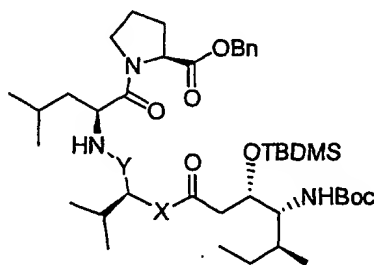
48. A method of making a didemnin fragment, the method comprising coupling a first reactant having the structure



and a second reactant having the structure



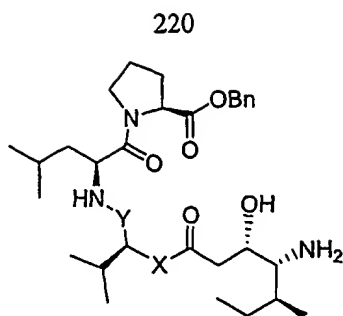
to yield a didemninn fragment having the structure



wherein X is selected from the group consisting of -O-, -NMe, and -NH-;  
where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

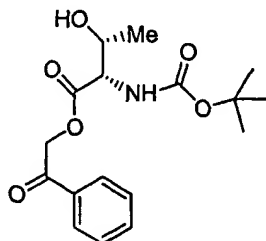
49. The method of claim 48, further comprising hydrolyzing the didemninn fragment to yield a didemninn fragment having the structure



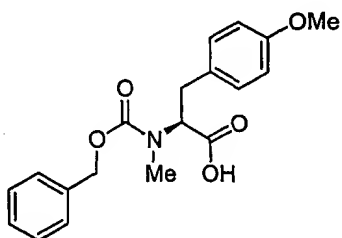


wherein X is selected from the group consisting of  $-O-$ , and  $-NH-$ ; where Y is  $-(COCHCH_3)_nCO-$ ; where n is 0 or 1.

50. A method of making a didetnnin fragment, the method comprising coupling a first reactant having the structure

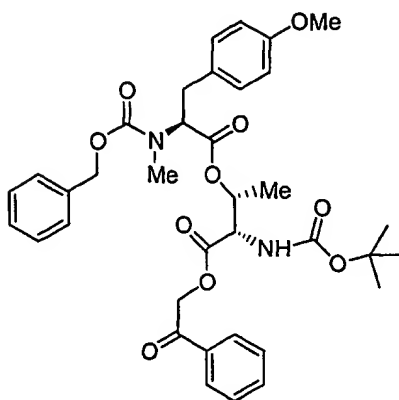


and a second reactant having the structure

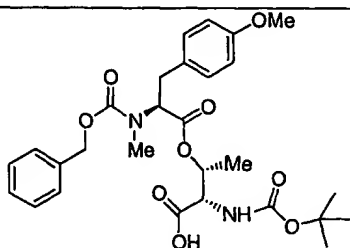


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to yield a didemninn fragment having the structure

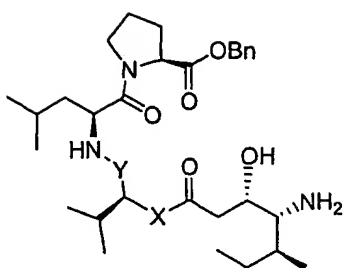


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51. The method of claim 50, further comprising deprotection of the benzyl ester group of the didemninn fragment to yield a didemninn fragment having the structure
- 

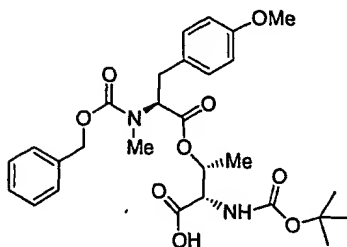


52. A method of making a didemninn fragment, the method comprising coupling a first reactant having the structure

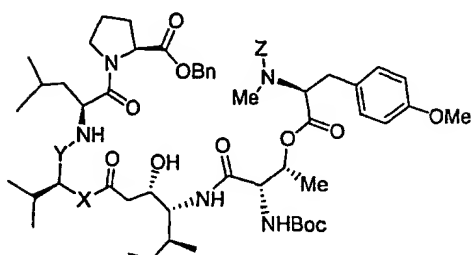
222



and a second reactant having the structure



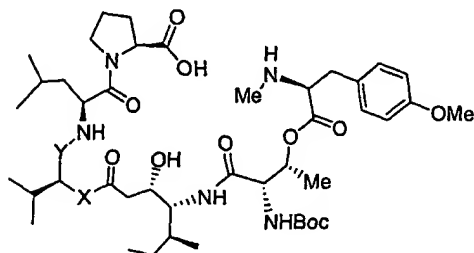
to yield a didemnin fragment having the structure



wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

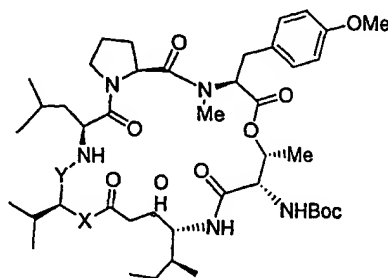
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53. The method of claim 52, further comprising deprotection the didemninn fragment to yield a didemninn fragment having the structure



wherein X is selected from the group consisting of  $-O-$ , and  $-NH-$ ; where Y is  $-(COCHCH_3)_nCO-$ ; where n is 0 or 1.

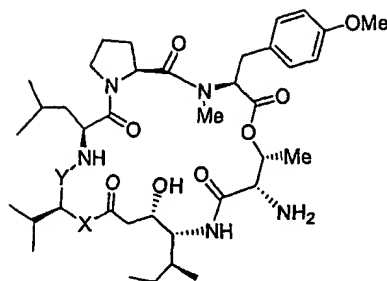
54. A method of making a didemninn fragment comprising the cyclizing the fragment of claim 53 to yield a didemninn analog having the structure



wherein X is selected from the group consisting of  $-O-$ , and  $-NH-$ ; where Y is  $-(COCHCH_3)_nCO-$ ; where n is 0 or 1.

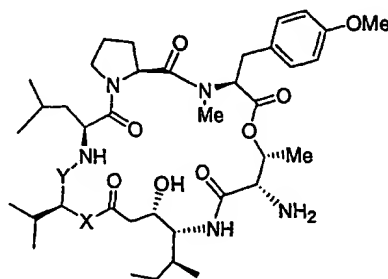
55. The method of claim 54, further comprising hydrolyzing the didemninn analog to yield a didemninn analog having the structure

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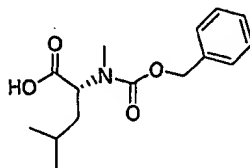


wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

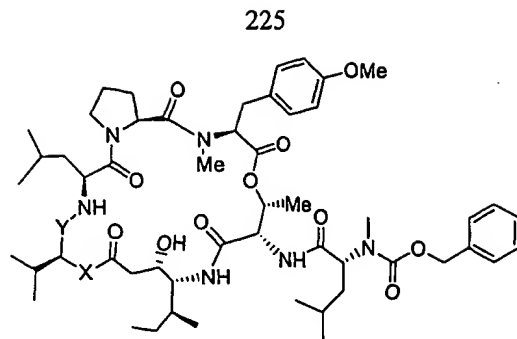
56. A method of making a didemnin analog, the method comprising coupling a first reactant having the structure



and a second reactant having the structure

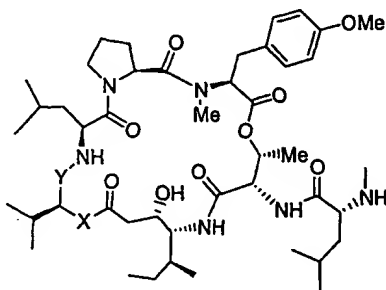


to yield a didemnin analog having the structure



wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

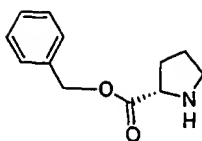
57. The method of claim 56, further comprising deprotection the didemninn fragment to yield a didemninn fragment having the structure



wherein X is selected from the group consisting of -O-, and -NH-; where Y is  $-(\text{COCHCH}_3)_n\text{CO}-$ ; where n is 0 or 1.

58. A method of making a didemninn fragment comprising the coupling of the fragment having the structure

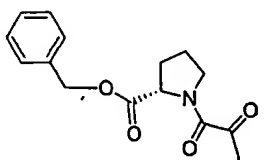
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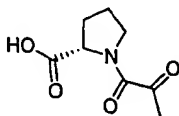
and a second reactant having the structure



to yield a didemnol fragment having the structure

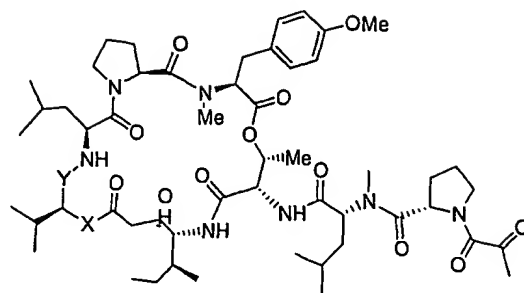


59. The method of claim 58, further comprising deprotection the didemnol fragment to yield a didemnol fragment having the structure



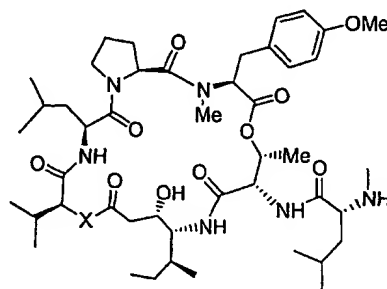
60. A method of making a didemnol analog comprising the coupling of the didemnol analog in claim 57 with the fragment in the claim 59, to yield the didemnol analog having the structure

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wherein X is selected from the group consisting of  $-O-$ , and  $-NH-$ ; where Y is  $-(COCHCH_3)_nCO-$ ; where n is 0 or 1.

61. A method of making a didemnins analog comprising the coupling of the didemnins analog having the structure



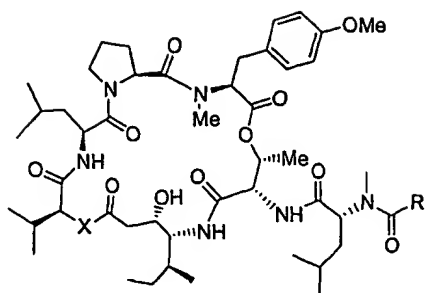
and the fragment having the structure



to yield the didemnins analog having the structure

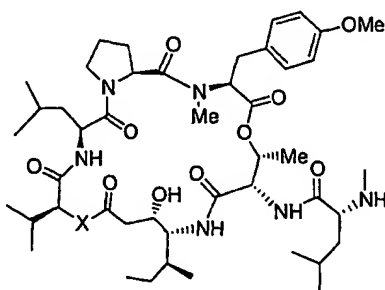


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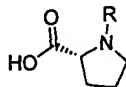


wherein X is selected from the group consisting of -O-, and -NH-, and R is i-Propyl; wherein X is -O- and R is n-Propyl, and R is n-Pentyl

62. A method of making a didemnins analog comprising the coupling of the didemnins analog having the structure

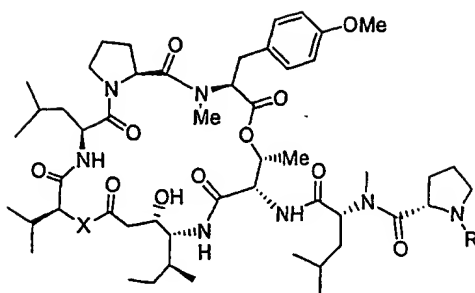


and the fragment having the structure

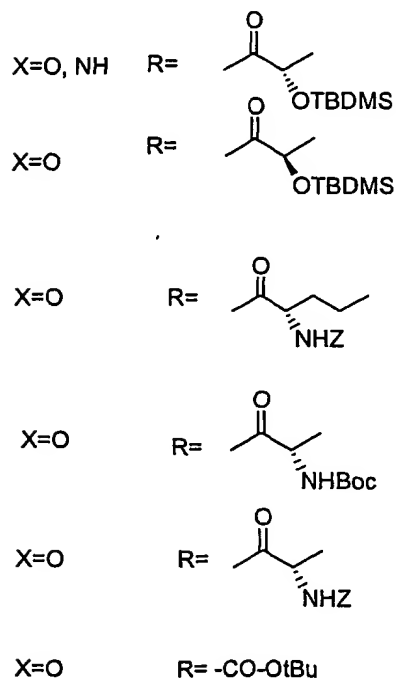


to yield the didemnins analog having the structure

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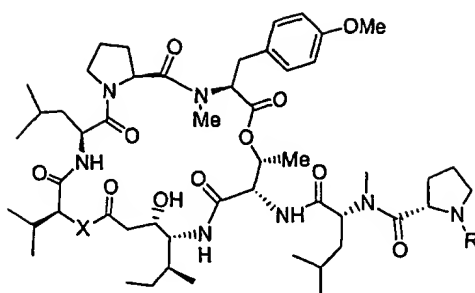


wherein :

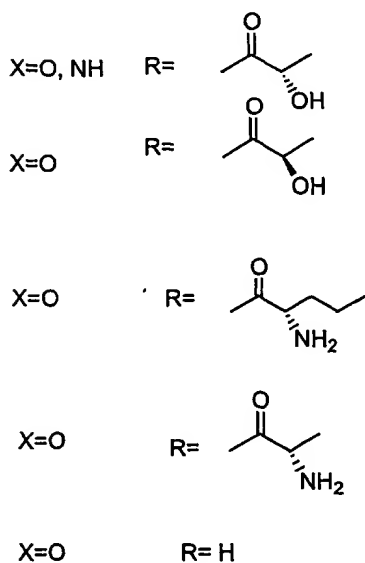


63. The method of claim 62, further comprising deprotection the didemnin analog to yield a didemnin fragment having the structure

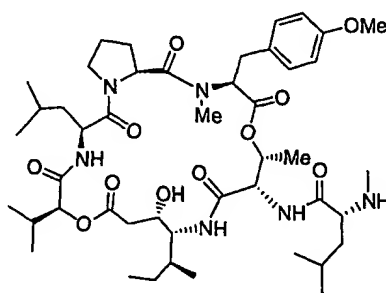
230



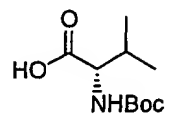
wherein



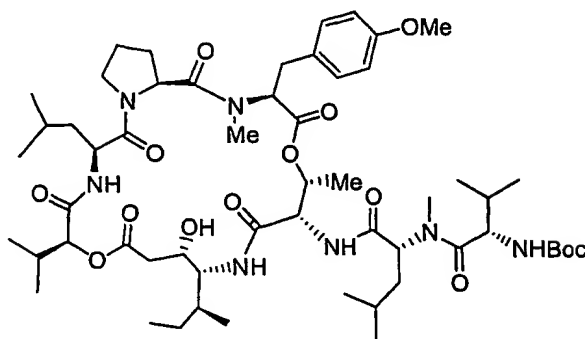
64. A method of making a didemninn analog comprising the coupling of the didemninn analog having the structure



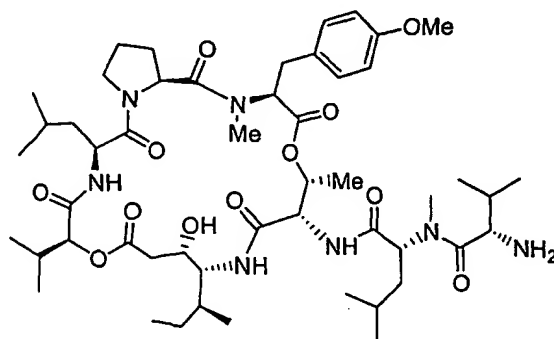
and the fragment having the structure



to yield the didemninn analog having the structure

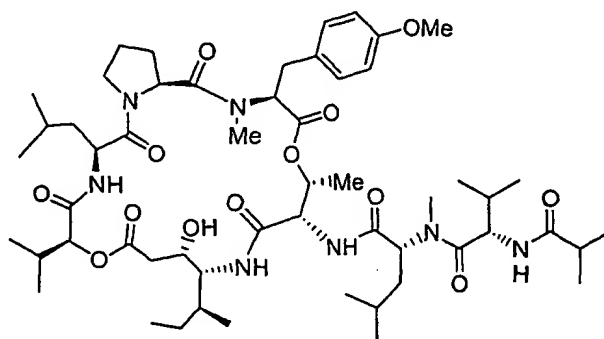


65. The method of claim 64, further comprising deprotection the didemninn analog to yield a didemninn analog having the structure

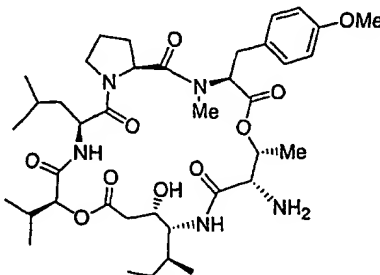


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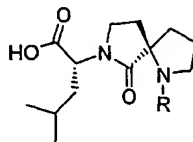
66. A method of making a didemninn analog comprising the coupling of the didemninn analog in claim 65, and isobutyryl chloride to yield the didemninn analog having the structure



67. A method of making a didemninn analog comprising the coupling of the didemninn analog having the structure

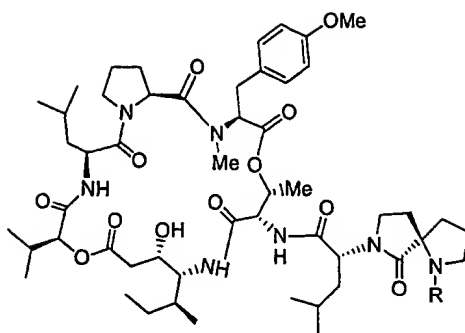


the fragment having the structure



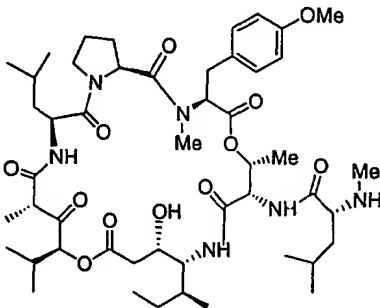
to yield the didemninn analog having the structure

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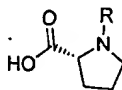


wherein R is Boc, isobutyryl, pyruvyl, or acriloyl.

68. A method of making a didemnin analog comprising the coupling of the didemnin analog having the structure

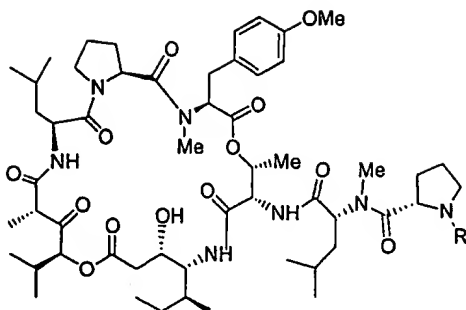


and the fragment having the structure



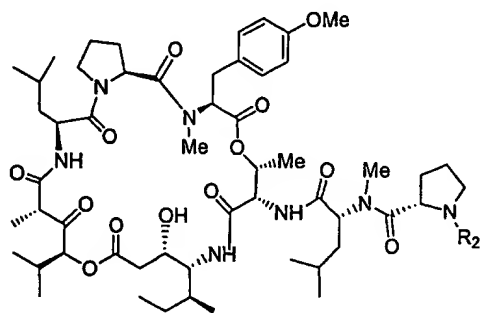
to yield the didemnin analog having the structure

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wherein R is SO<sub>2</sub>Me, and Z-Nva.

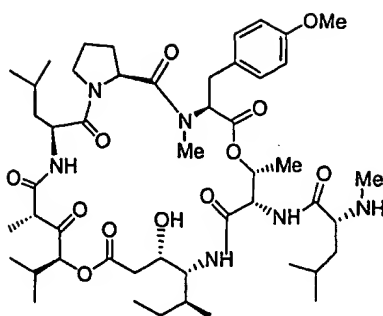
69. The method of claim 68, further comprising deprotection the didemnin analog to yield a didemnin analog having the structure



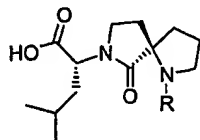
wherein R<sub>2</sub> is Nva.

70. A method of making a didemnin analog comprising the coupling of the didemnin analog having the structure

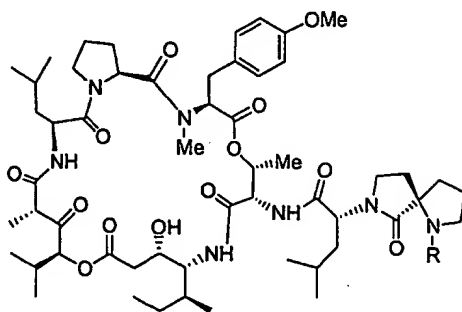
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and the fragment having the structure



to yield the didemnins analog having the structure

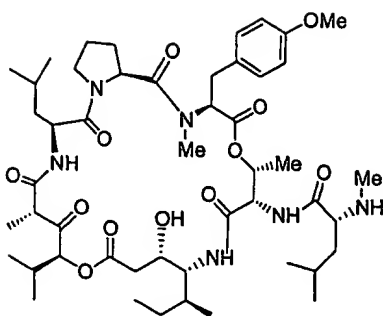


wherein R is Boc, isobutyryl, or pyruvyl.

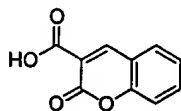
71. A method of making a didemnins analog comprising the coupling of the didemnins analog having the structure



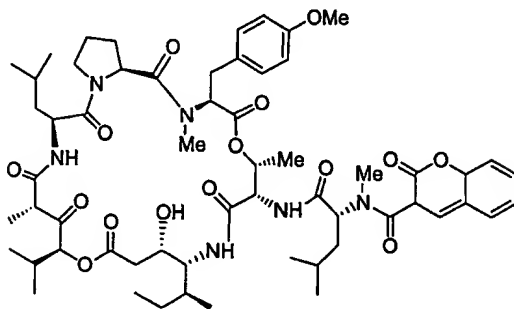
236



and the fragment having the structure

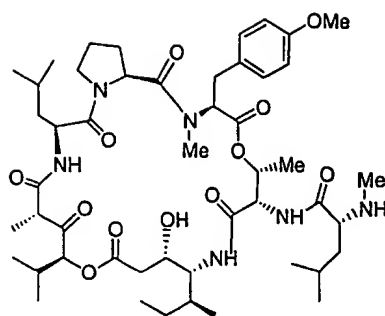


to yield the didemnins analog having the structure

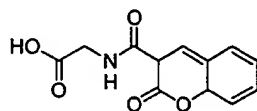


72. A method of making a didemnin analog comprising the coupling of the didemnin analog having the structure

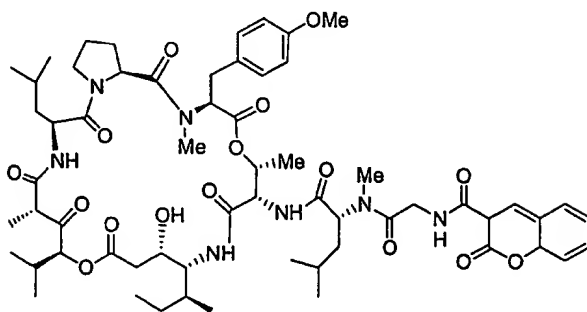
237



and the fragment having the structure

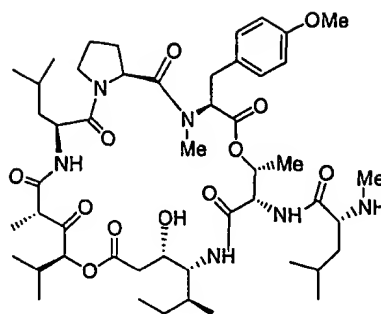


to yield the didemnins analog having the structure

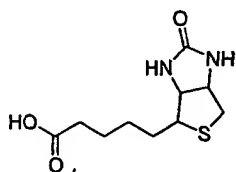


73. A method of making a didemnin analog comprising the coupling of the didemnin analog having the structure

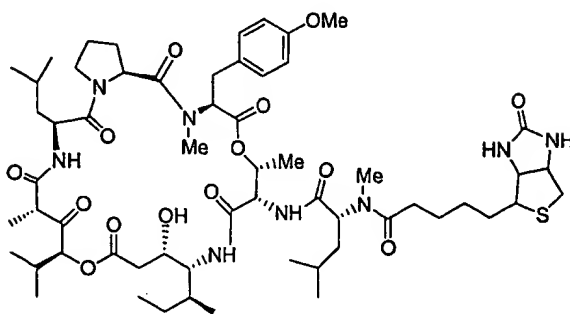
238



and the fragment having the structure

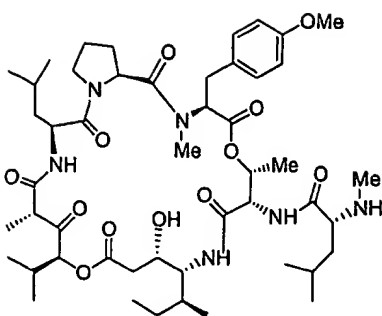


to yield the didemnyn analog having the structure

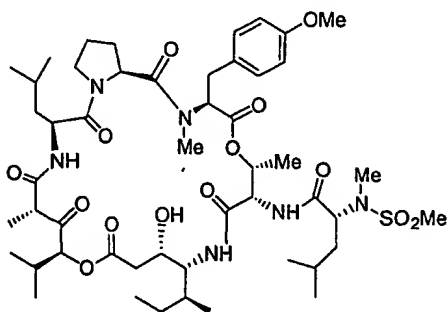


74. A method of making a didemnin analog comprising the coupling of the didemnin analog having the structure

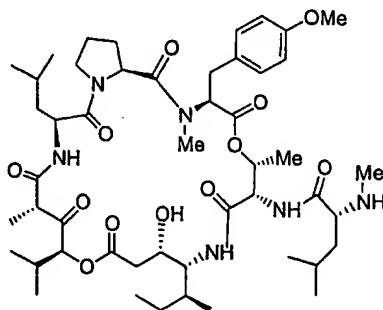
239



and methylsulphonyl chloride, to yield the didemnin analog having the structure

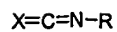


75. A method of making a didemnin analog comprising the coupling of the didemnin analog having the structure

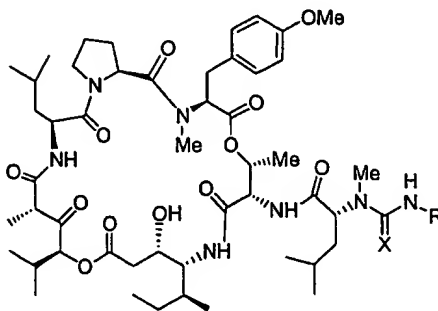


and the fragment having the structure

240



to yield the didemnyn analog having the structure



wherein X is O, and S; wherein R is butyl, and phenyl.

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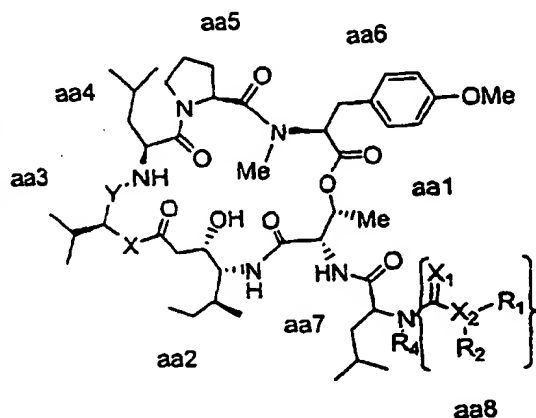
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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: **SYNTHETIC METHODS FOR APLIDINE AND NEW ANTITUMORAL DERIVATIVES, METHODS OF MAKING AND USING THEM**

WO 02/02596 A3



(57) Abstract: The invention provides aplidine derivatives and synthetic methods. They are represented by the formula: where the different symbols have the meaning indicated in the description.

# INTERNATIONAL SEARCH REPORT

International Application No

PC1/GB 01/02901

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K11/00 C07K11/02 C07C271/22 A61K38/04

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

WPI Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 98 17302 A (UNIVERSITY OF ILLINOIS) 30 April 1998 (1998-04-30) the whole document ---	1-32
X	WO 98 17275 A (UNIVERSITY OF ILLINOIS) 30 April 1998 (1998-04-30) the whole document ---	1-75
X	WO 93 00362 A (BASF AG) 7 January 1993 (1993-01-07) the whole document ---	1-32
X	EP 0 393 883 A (UNIVERSITY OF ILLINOIS) 24 October 1990 (1990-10-24) the whole document --- -/--	1-32

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## INTERNATIONAL SEARCH REPORT

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X	US 6 034 058 A (RINEHART ET AL.) 7 March 2000 (2000-03-07) the whole document ---	1-32
A	R SAKAI ET AL.: "Structure-activity relationship of didemnins" JOURNAL OF MEDICINAL CHEMISTRY., vol. 39, no. 14, 1996, pages 2819-2834, XP002943723 AMERICAN CHEMICAL SOCIETY. WASHINGTON., US ISSN: 0022-2623 cited in the application the whole document ---	35-75
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A	ES 2 102 322 A (PHARMA MAR SA) 16 July 1997 (1997-07-16) the whole document ---	35-75
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Information on patent family members

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